

RECYT

Year 24 / Nº 37 / 2022 / 73–80

DOI: <https://doi.org/10.36995/j.recyt.2022.37.009>

## Genetic and epi-genetic alterations of the PTEN gene: key in the relationship between type 2 diabetes and cancer

### Alteraciones genéticas y epi-genéticas del gen PTEN: clave en la relación entre diabetes mellitus tipo 2 y cáncer

Alan Rolando Ayala Schimpf<sup>1,2</sup>, María Mercedes Formichela<sup>4,5</sup>, María Betania Mascheroni<sup>5,6</sup>, Donovan Rivero<sup>1,2</sup>, Marcela Luján Chamorro<sup>5</sup>, Valeria Portillo<sup>6</sup>, Pedro Darío Zapata<sup>1,2</sup>, Cristian Alberto Ferri<sup>1,3,\*</sup>

1- Universidad Nacional de Misiones. Facultad de Ciencias Exactas Químicas y Naturales. Instituto de Biotecnología Misiones "Dra. María Ebe Reza" (INBIOMIS). Laboratorio de Biotecnología Molecular (BIOTECMOL). Misiones, Argentina.

2- CONICET. Buenos Aires, Argentina.

3- Instituto Universitario de Ciencias de la Salud, Fundación Héctor A. Barceló, Facultad de Medicina, Sede Santo Tomé, Centeno 710. Corrientes, Argentina.

4- Cátedra de Bioquímica Clínica II. Módulo de Farmacia y Bioquímica, Facultad de Ciencias Exactas Químicas y Naturales, Universidad Nacional de Misiones. Av. Mariano Moreno 1375 (3300) - Posadas, Misiones, Argentina.

5- Laboratorio CEBAC, Cede de la Clínica Nosiglia, Posadas, Misiones, Argentina.

6- Centro de Medicina Preventiva (PREDIGMA), Posadas, Misiones, Argentina.

\* E-mail: [cristianferri02@gmail.com](mailto:cristianferri02@gmail.com)

Received: 14/06/2021; Accepted: 15/10/2021

#### Abstract

Cancer pathogenesis has been associated with genetic and epigenetic alterations that may lead to the inactivation or decrease of tumor-suppressor genes, such as the *PTEN* gene. In particular, alterations in the *PI3K/PTEN* signaling pathways are related to abnormalities associated with diabetes, metabolic syndrome, obesity and cardiovascular diseases. The aim of the study was to evaluate whether the expression levels of the *PTEN* gene, the 32-bp deletion in the *PTEN* gene, and the methylation in the *PTEN* promoter are associated with Type 2 Diabetes Mellitus (T2DM) and cancer development. Peripheral blood samples were obtained from a total of 70 patients of both sexes: 40 patients with T2DM and 30 patients with T2DM and cancer (T2DM+C). In this a novel report that assesses the upregulation of *PTEN* in T2DM, we observed that *PTEN* expression is higher in T2DM patients than in healthy individuals and markedly lower in T2DM patients with cancer than in T2DM patients without cancer. Hypermethylation of the *PTEN* promoter is more frequent in T2DM patients with cancer. Genetic and epigenetic alterations of the *PTEN* gene may be a key in the relationship between T2DM and cancer. *PTEN* expression may serve as a potential candidate biomarker for cancer development in T2DM patients.

Keywords: *PTEN* expression; Type 2 Diabetes Mellitus; Cancer; Hypermethylation.

#### Resumen

La patología del cáncer ha sido asociada con alteraciones genéticas y epigenéticas que pueden llevar a la inactivación o subexpresión de genes supresores de tumores, como el gen *PTEN*. Las alteraciones en las vías de señalización de *PI3K/PTEN* están relacionadas con anomalías asociadas a diabetes, síndrome metabólico, obesidad y enfermedades cardiovasculares. El objetivo del estudio fue evaluar, si la expresión alterada (subexpresión/sobreexpresión) del gen *PTEN*, la delección de 32 pb y la metilación del promotor, se asocian con diabetes mellitus tipo 2 (DMT2) y el desarrollo de cáncer. Las muestras de sangre periféricas se obtuvieron de un total de 70 pacientes de ambos sexos: 40 pacientes con DMT2 y 30 pacientes con DMT2 y cáncer (DMT2+C). En este trabajo novedoso, se reporta la sobreexpresión de *PTEN* en DMT2. Observamos que la expresión de *PTEN* es más alta en pacientes con DMT2 que en individuos sanos y marcadamente menor en pacientes con DMT2+C, en comparación con los pacientes diabéticos sin neoplasias. La hipermetilación del promotor de *PTEN* es más frecuente en pacientes con DMT2 y cáncer. Las alteraciones genéticas y epigenéticas en el gen *PTEN* podría ser clave en la relación existente

entre diabetes y el desarrollo de cancer. La expresión de *PTEN* puede ser un potencial biomarcador en el desarrollo cancer en pacientes con DMT2.

Palabras clave: Expresión de *PTEN*; Diabetes Mellitus Tipo 2; Cáncer; Hipermetilación.

## Introduction

Cancer pathogenesis has been associated with genetic and epigenetic alterations that may lead to the inactivation or decrease of tumor-suppressor genes<sup>1</sup>. Among these, the phosphatase and tensin homolog (*PTEN*) gene is frequently deleted or inactivated in various tumors, suggesting that the loss of a single *PTEN* allele is sufficient to drive tumorigenesis<sup>2,3</sup>. It has been reported that minimal changes in the expression level of *PTEN* can have profound effects on tumor susceptibility<sup>4</sup>. In addition, mechanisms such as methylation or mutations in the gene promoter may lead to a loss of *PTEN* function<sup>5,6</sup>. This loss of *PTEN* function is involved in the development of cancer and other human diseases<sup>7-13</sup>. In addition, polymorphisms in the *PTEN* gene have been associated with Type 2 Diabetes Mellitus (T2DM)<sup>14-18</sup>.

T2DM is closely related to obesity and both are characterized by insulin resistance<sup>19</sup>, generally defined as a reduction in the ability of insulin to exert its metabolic effects on tissues<sup>20</sup>. Although the etiology of T2DM can be very different, both have been related to alterations in insulin secretion, in the sensitivity to the action of insulin, or both<sup>21-23</sup>. Since insulin is also a powerful promoter of cell growth, it has been involved in the development of some types of cancer<sup>24,25</sup>. In fact, a strong relation between T2DM and the risk of developing certain types of cancer has been reported<sup>25-27</sup>.

Insulin action activates two main signal transduction pathways: the phosphatidylinositol-3-kinase (PI3K) pathway and the mitogen-activated protein kinase (MAPK) pathway. Both pathways in turn regulate most insulin actions associated with the regulation of energy metabolism and gene expression<sup>28-30</sup>. In muscle tissue and adipocytes, the metabolic actions induced by insulin, including GLUT4 translocation, glucose uptake and glycogen synthesis, in turn depend on the activation of another pathway, the PI3K/Akt pathway<sup>14,31</sup>. In addition, it has been demonstrated that abnormalities associated with diabetes, metabolic syndrome, obesity, hypertension and cardiovascular diseases are related to the PI3K/*PTEN* signaling pathway<sup>2,30,32-35</sup>.

Thus, since T2DM and cancer are connected and *PTEN* may be a crucial gene related to both diseases, we consider it is essential to evaluate the *PTEN* expression pattern in T2DM patients and T2DM patients with cancer. Based on this, the aim of the present study was to evaluate *PTEN* expression, *PTEN* methylation and the frequent 32-bp deletion in the *PTEN* gene (known as rs34421660 genetic variant) and their relationship with T2DM and cancer development.

## Materials and methods

### Patients

To carry out this work, we have performed a case control study. All the participants provided an informed consent and their anonymity was preserved. The study was approved by the Ethics Committee of Dr. Fernando Barreyro Hospital, Posadas, Misiones, Argentina. A total of 135 samples were analyzed. Peripheral blood samples were obtained from 70 patients of both sexes, aged between 17 and 83 years old: 40 patients with T2DM and 30 patients with T2DM and cancer (T2DM+C). All samples were obtained at CEBAC laboratories of the Nosiglia Clinic, Posadas, Misiones, Argentina. Control samples were collected from 65 healthy individuals of both sexes, aged between 18 and 80 years old, all from Posadas.

### Sample preparation

Total RNA and DNA were extracted from total leukocyte cells from peripheral using Triagent® following the manufacturer's protocol. First-strand cDNA synthesis was performed with random hexamer primers and M-MLV reverse transcriptase®.

### Polymorphism detection

32-base-pair (bp) deletion polymorphism (rs34421660) was detected by conventional PCR, using Taq DNA polymerase 500U InBio Highway®. We obtain a fragment of 209 bp or 241 bp depending if the deletion was present or not. The primers used have been previously published<sup>36</sup> (Table 1). The reactions were carried out in the thermocycler Bioer GenePro. The rs34421660 polymorphism was corroborated by direct sequencing, using the PCR product corresponding to homozygous cases, one with deletion and the other without it (Figure 1). The PCR product was electrophoresed on 2% agarose gel containing ethidium bromide, a band corresponding to the expected size was purified using GFX columns (GE Healthcare) and then sequenced.

### *PTEN* promoter methylation

The DNA samples were treated with sodium bisulfite; while methylated C residues resisted the treatment. Stanganeli *et al.*<sup>37</sup> method was used for the bisulfite treatment of DNA with certain modifications. Briefly, 1 µg of genomic

DNA was denatured with 3 M NaOH at 37°C for 20 min and modified with 4.5 M sodium bisulfite (Sigma, St. Louis, MO, USA) and 10 mM hydroquinone (Sigma, St. Louis, MO, USA) at 55°C for 5 h. After purification with the Wizard™ DNA Clean-up System (Promega A7280, Madison, WI, USA), the DNA was desulfonated in 3 M NaOH. The subsequent PCR amplification was performed using primers designed previously for either methylated or unmethylated DNA<sup>36,38</sup> (Table 1). The forward and reverse primers used for the first stage allowed amplify a fragment of 529 pb amplicon; 5 µl of the first-stage PCR product was used as a template for the second PCR stage. In the second stage, if the amplification product was unmethylated, the amplicon obtained was 173 bp, whereas, if the product was methylated, the amplicon obtained was 155 bp. Due to the difficulty in the process of methylation and subsequent amplification, only 42 samples could be analyzed by methylation of the *PTEN* promoter.

Thermal profiles consisted of an initial denaturation step at 95°C for 5 min, followed by 35 cycles at 97 °C for 45s, 58 °C for 30s, and 72 °C for 60 s, with a final extension step of 10 min at 72 °C. Reaction products were visualized after electrophoresis on 2% agarose gels (Tris-acetate EDTA

buffer, pH 8) containing ethidium bromide.

### ***PTEN* expression**

To observe *PTEN* gene expression, Quantitative PCR was carried out using BIOER -Line Gene K Real Time, FDQ-48A (A4), as follows: 95 °C for 3 min, 50°C for 1 min, followed by 40 cycles to 95°C for 15 s and 60 °C for 1 min. The melting curve analysis and gel electrophoresis experiments were used to ensure that only one product of the expected size was amplified. Non-template controls were included in each run. Expression values were obtained from the threshold cycle (Ct value), using serial dilutions of the K562 cell line as standard curve.

The expression of the *PTEN* gene was normalized by subtracting the Ct value of the housekeeping gene (*β-actin*) from the Ct value of the target gene ( $\Delta Ct$ ). The relative copy number of *PTEN* mRNA was calculated according to  $2^{-\Delta Ct}$ , and then expressed as a percentage. *PTEN* expression was measured in duplicate for each sample. The primers (table 1) and conditions have been previously published<sup>39</sup>.

### **Statistical analysis**

The association between the rs34421660 polymorphism and diabetes and cancer was analyzed by univariate Fisher's exact test and multivariable logistic regression analysis. The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated. The ORs were analyzed with the use of the SPSS software (version 15.0) (IBM, SPSS Inc., Chicago, USA), and Hardy-Wein-

berg equilibrium (HWE) was tested using a goodness-of-fit Chi-square test. Standard genetic models (additive, recessive, co-dominant and dominant) for disease penetrance were evaluated. Differences in expression levels were analyzed using one-way analysis of variance (ANOVA) and an unpaired *t* test to compare differences between groups. The statistical analyses were performed using GraphPad Prism5.0. All statistical tests were two-sided and values of  $p \leq 0.05$  were considered statistically significant. To determine a value allowing the identification of cases with increased *PTEN* expression with respect to the healthy individuals (HI), a cut-off value from the healthy group was calculated as follows: mean + 3 standard deviation (SD)<sup>39</sup>.

## **Results**

### **Case-control study**

Out of the 135 individuals incorporated in this study, 70 had T2DM and 30 of these individuals were also diagnosed with cancer (testicle, breast, colon, lung, prostate, kidney and pancreas). The distribution regarding gender and age is shown in Table 2.

The rs34421660 genotype frequencies were also analyzed comparing patients and controls according to DM2, DM2+C and the Control Group (Table 3). Genotype and allele frequency distributions for rs34421660 in cases and controls are shown in Table 4.

No deviation from HWE was found either for controls or patients ( $p=0.38$  and  $p=0.45$ , respectively).

**Table 1:** Sequences of all PCR primers used in this study.

Primers used	primers' sequence (5'-3')
PTEN sequencing primers	FW: CCAGCCCTCACTAAAAACAAA
	Rv: CAAGTGCCAAGCAGCAAA
Methylation primers	FW: TTTAGGGAGGGGTTTGA
	Rv: CCATCCTCTAATATCTCCT
	FW: GGTTCGGAGGTCGTCGGC
	Rv: CAACCGAATAATAACTACTACGACG
PTEN expression primers	FW: TGGGTTTTGGAGGTTGTTGGT
	Rv: ACTTAACCTAAACCACAACCA
PTEN expression primers	FW: CAGTCAGAGGCGCTATGTGTATTA
	Rv: AACTTGCTCTCCCGTCGTGTG
<i>β-actin</i> primers	FW: CCAGAGGCGTACAGGGATAG
	Rv: CCAACCGCGAGAAGATGA

**Table 2:** Patients: distribution by gender and age.

Population	Female	Male	Median (range)
HI	38	27	54 (18-80)
T2DM	16	24	64 (18-86)
T2DM+C	13	17	58 (22-83)
TOTAL	67	68	135

HI: Healthy individuals. T2DM: patients with Type 2 Diabetes Mellitus. T2DM+C: patients with Type 2 Diabetes Mellitus and cancer

**Table 3:** Distribution of the rs34421660 variant in T2DM, T2DM+C and Control Group.

Patients	Genotypes			OR
	ins/ins (%)	ins/del (%)	del/del (%)	
Control (n=65)	33 (50.77)	24 (36.93)	8 (12.30)	1.248 (0.569-2.802)
T2DM (n=40)	19 (42.85)	15 (37.15)	8 (20)	
Control (n=65)	33 (50.77)	24 (36.93)	8 (12.30)	2.06 (0.836-5.305)
T2DM+C (n=30)	10 (33.34)	16 (53.33)	4 (13.33)	
T2DM (n=40)	19 (42.85)	15 (37.15)	8 (20)	1.652 (0.638-4.111)
T2DM+C (n=30)	10 (33.34)	16 (53.33)	4 (13.33)	

T2DM: Type 2 diabetes mellitus. T2DM+C: Type 2 diabetes mellitus and cancer. ins: Without 32 bp deletion. del: correspond to 32 bp deletion.

**Table 4:** Association study between the rs34421660 polymorphism and diabetes risk.

Allele†	Genotype	Control %	Patients %	OR (95% CI)	P-value
	ins	82 (71)	78 (64)	1(ref)	
del	34 (29)	44 (36)			
<b>Genetic model‡</b>					
Codominant	ins/ins	33 (50.8)	28 (40.0)	1 (ref)	0.54
	ins/del	24 (36.9)	30 (42.9)	1.33 (0.51-3.46)	
	del/del	8 (12.3)	12 (17.1)	2.04 (0.56-7.47)	
Dominant	ins/ins	33 (50.8)	28 (40.0)	1(ref)	0.36
	ins/del-del/del	32 (49.2)	42 (60.0)	1.51 (0.63-3.62)	
Recessive	ins/ins-ins/del	57 (87.7)	58 (82.9)	1(ref)	0.34
	del/del	8 (12.3)	12 (17.1)	1.79 (0.53-6.05)	
Overdominant	ins/ins-del/del	41 (63.1)	40 (57.1)	1(ref)	0.82
	ins/del	24 (36.9)	30 (42.9)	1.11 (0.45-2.71)	
Log-additive				1.41 (0.76-2.58)	0.27

†Univariate analysis by Fisher's exact test. ‡Logistic regression adjusted by sex and age.

### Detection of the rs34421660 polymorphism (32-bp deletion)

After PCR amplification, all the samples were cataloged based on whether the rs34421660 polymorphism was present or absent considering gender and condition (patients or controls). The distribution of rs34421660 by condition is shown in Table 3.

The rs34421660 polymorphism seemed not to be associated with the development of diabetes [OR: 1.825 (0.804-4.140)] or cancer [OR: 1.64 (0.599-4.494)] and not involved in different levels of *PTEN* expression ( $p=0.45$ ).

### *PTEN* expression

The ANOVA ( $\alpha=0.05$ ) showed statistical differences in the *PTEN* expression levels ( $p<0.0001$ ). Statistical parameter values of *PTEN* expression in the populations analyzed were as follows: statistical mean ( $\bar{x}$   $\bar{x}$  + HI: 3.69;  $\bar{x}$   $\bar{x}$  + T2DM: 24.25;  $\bar{x}$   $\bar{x}$  + T2DM+C: 7.74); SD  $\sigma$  ( $\sigma$  HI: 4.58;  $\sigma$  T2DM: 13.11;  $\sigma$  T2DM+C: 7.08); and standard

error (SE) (SE HI: 0.57; SE T2DM: 2.62; SE T2DM+C: 1.67). The *t* test analysis was performed with significant level of  $\alpha=0.05$  and patients were compared between them and with HI. *PTEN* expression was statistically higher in T2DM patients than in HI ( $p<0.0001$ ) and T2DM+C patients ( $p<0.0001$ ). Besides, *PTEN* expression in T2DM+C patients was statistically different from that in HI ( $p=0.0045$ ) (Figure 2).

To determine a value that allow the identification of cases with increased *PTEN* expression with respect to HI, a cut-off value from the HI group was calculated as follows: mean + 3SD:  $3.69 + (3 \times 4.58) = 17.43$ . Unlike that observed in the other groups, 68% of T2DM patients presented an overexpression of the *PTEN* gene (Figure 3).

### Methylation of the *PTEN* promoter

The methylation status of the *PTEN* promoter was analyzed in 42 cases of the three groups (Table 5). T2DM+C patients showed higher frequency of methylation than T2DM patients and HI ( $p=0.0215$ ).

The first PCR stage, which produced a 529-bp amplicon, was used as a template for the second PCR stage, and the second stage was performed as previously described 40, 41. In the second stage, the unmethylated amplification product was a 173-bp fragment, whereas the methylated amplification product was a 155-bp fragment.

**Table 5:** Methylation status of the *PTEN* gene promoter.

Population	N° of cases analyzed	Methylation status		
		MET	MET/UNM	UNM
HI	15	0	11	4
T2DM	15	0	15	0
T2DM+C	12	9	3	0

HI: Healthy individuals, T2DM: patients with Type 2 Diabetes Mellitus, T2DM+C: patients with Type 2 Diabetes Mellitus and cancer.

MET: methylated; UNMET: unmethylated

### Discussion

*PTEN* is a protein phosphatase with dual activity, both involved in the insulin signaling pathway and working as a tumor suppressor 42. The metabolic action of insulin depends on the PI3K signaling pathways, and some defects in these pathways have been reported in T2DM patients. In addition, *PTEN* can negatively regulate the positive actions of PI3K through the regulation of PIP3 levels 43, 44. Thus, this negative regulation could "improve" the insulin signaling. Although the *PTEN* tumor-suppressor functions have already been widely demonstrated, the physiological role of *PTEN* in glucose metabolism is largely unknown.

Currently few studies are focusing on *PTEN* as a possible mediator by way of suppressor tumor, as well as negative regulator at the front desk of the insulin at the cellular level and its involvement in the development of

cancer in individuals diagnosed with T2DM.

The aim of this work was to characterize the 32-bp deletion known as rs34421660 polymorphism in the *PTEN* gene and its association with the susceptibility to develop cancer in patients with T2DM. Besides, the levels of gene expression were assessed in patients whose PI3K pathway could be altered due to T2DM, and compared with respect to the expression levels in T2DM patients with cancer, considering *PTEN* functions as a tumor suppressor.

Polymorphisms can either be silent (and thus have no direct functional repercussions) or provide advantages or disadvantages against certain diseases in individuals carrying them<sup>45,46</sup>. Researches aimed to identify frequent genetic polymorphisms that confer susceptibility to suffer a particular disease are becoming more frequent<sup>47,48</sup>. If these polymorphisms are present in the promoter region, they might derive in an altered gene expression. Similarly, when the changes occur in intronic regions, they could modulate the gene expression. In this context, specific molecular studies can demonstrate whether polymorphisms are functional, and epidemiological studies are fundamental to assess whether their presence is a risk factor for population health<sup>49</sup>. In this study, no statistically significant association was observed between the rs34421660 polymorphism and T2DM and/or cancer development in T2DM patients. The role of *PTEN* as a tumor suppressor and its expression levels are regulated by several and complex mechanisms<sup>41</sup>. The physiological action of *PTEN* is to block PI3K signaling by PIP3 dephosphorylation, avoiding related effects by PIP3 increase, as AKT signaling deregulation<sup>42,50</sup>. An increase in AKT activity leads to amplified cell survival, growth and proliferation<sup>51</sup>. It has been reported that patients diagnosed with many types of neoplasms have a lower expression of *PTEN* than those without neoplasms. These investigations are based on the development of cancer as the final result of the deregulation of the cell cycle and apoptosis inhibition<sup>52,53</sup>. *PTEN* modulates the metabolic actions in the cell by regulating the pathway downstream PI3K, where, in addition to reducing the levels of PIP3, it can also dephosphorylate the insulin receptor substrate, altering the signaling of the insulin PI3K/AKT pathway through these two mechanisms<sup>54</sup>. An increase in *PTEN* expression, and therefore in its activity, should lead to the same impact on the signaling of insulin as a decrease in the activity of the PI3K pathway<sup>55</sup>.

In this study, the expression of *PTEN* presented a marked variation in the T2DM population with respect to healthy individuals. Through the cut-off value, calculated from the *PTEN* expression in healthy individuals, we identified that 68% of the T2DM patients studied had *PTEN* overexpression. A recent study related to the expression of this gene and its association with T2DM in a population of a region of Western China has recently found that this expression would be related to severe insulin resistance<sup>6</sup>. In addition, *PTEN* is involved in the control of muscle

protein metabolism, presenting a fluctuating behavior in catabolic conditions, and it has been shown that high levels of *PTEN* expression contribute to the degradation of muscle proteins, a characteristic phenomenon in T2DM<sup>56</sup>. The T2DM mechanism that would lead to muscle atrophy is related to the reduction of protein synthesis. Proteolysis of the muscle in catabolic conditions is linked to insulin resistance and specifically to the defects in the substrate of the insulin receptor associated with the PI3K/AKT pathway. Experimental evidence has shown that *PTEN* levels vary with models of defects in insulin signaling, and that *PTEN* suppression produces a marked improvement of glucose concentrations in blood and of the insulin sensitivity in diabetic mice. On the other hand, overexpression of *PTEN* inhibits glucose uptake and GLUT4 translocation<sup>57</sup> and a high-fat diet-induced insulin resistance increases the activity of *PTEN*<sup>58</sup>. The *PTEN* overexpression in patients with T2DM observed in this study is consistent with previous experimental evidence suggesting that *PTEN* is transcriptionally regulated in insulin chronic deficiency/resistance in T2DM. The mechanism of increased *PTEN* expression observed in T2DM would mean an increase in *PTEN* mRNA. The activity and expression of *PTEN* can be increased by the incubation of endothelial cells with free fatty acids. In this case, *PTEN* overexpression was related to the stimulation of p38 MAPK<sup>59</sup>. Findings in muscle cells in culture have confirmed not only that palmitic acid increases p38 activation and *PTEN* expression, but also that p38 inhibition suppresses the increase in palmitic acid in *PTEN* mRNA and the expression of induced to protein. These results suggest that the transcription and expression of *PTEN* are related to the activation of p38, which could partly explain the high expression of *PTEN* in the T2DM population<sup>59,60</sup>.

In many tumors, the *PTEN* gene is mutated or deleted<sup>42</sup>. Although in the present study we expected that T2DM patients with cancer would show decreased *PTEN* expression levels, we found no statistically significant difference with respect to those of the healthy individuals studied. Due to the great overexpression of *PTEN* observed in T2DM patients and considering that the diagnosis of cancer in the T2DM+C patients was subsequent to the T2DM condition, it is necessary to compare these two populations together. Although there was statistical difference selection to compare the levels of *PTEN* in T2DM patients with cancer and healthy individuals, the levels of expression in T2DM patients with cancer were markedly lower ( $p > 0.0001$ ) than those in T2DM patients without cancer. This may allow us to infer that, during T2DM, given the need to maintain over-expressed cellular homeostasis values of *PTEN*, variations in the levels of expression could lead to a deregulation of the “balance” reached in signaling pathways and promote the development of neoplasms. To prove this, it would be necessary to perform a prospective study in T2DM patients that develop any neoplasia over

time and show decreased expression of *PTEN*. In addition to mutations in the *PTEN* gene, gene silencing through the hypermethylation of its promoter has been suggested as a potential mechanism that contributes to the down-regulation of the PI3K pathway by PTEN, which, at the same time, is positively associated with an increased risk to several types of cancer. This relationship between aberrant methylation and cancer has become evident by the fact that the absence of methylation leads to the activation of oncogenes and the presence of methylation annuls the protective action of tumor-suppressor genes. In the present study, we evaluated the methylation profile of the *PTEN* promoter in the different populations and established its association with the low expression of *PTEN* in T2DM patients with cancer. The inactivation of tumor-suppressor genes contributes to carcinogenesis by conferring certain advantages to indefinite cell growth, which is consistent with the partial results here obtained.

## Conclusion

The rs34421660 polymorphism of *PTEN* in intron 2 was present in 44% of the healthy individuals studied. This polymorphism seems not to be associated with the development of T2DM or cancer as well as not involved in different levels of *PTEN* expression. However, *PTEN* expression levels are higher in T2DM patients than in healthy individuals and markedly lower in T2DM patients with cancer than in T2DM patients without cancer. The hypermethylated *PTEN* promoter is more frequent in T2DM patients with cancer. Genetic and epigenetic mechanisms of the *PTEN* gene may be a key in the relationship between T2DM and cancer. *PTEN* expression may serve as a potential candidate biomarker for cancer development in T2DM patients.

## Acknowledgments

This study was supported in part by grant from the National Agency of Scientific and Technical Promotion (SECyT-PICT).

Thanks to Professor Lic. Ma. Victoria Gonzalez Eusevi for editing and proofreading the article.

## Disclosure

The authors declare no conflict of interests.

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## Legend to Figures

**Figure 1:** A: Representative polymerase chain reaction (PCR) products resolved by agarose gel electrophoresis to detect the presence or absence of the rs34421660 polymorphism (32-bp deletion) of the *PTEN* gene. M, DNA marker. Lane 1, heterozygous 32-bp deletion; lane 2: homozygous 32-bp deletion; lane 3: homozygous non-32-bp deletion. B: Genotype patterns for 32-bp homozygous deletion.

**Figure 2: Percentage of the mean of PTEN expression.** PTEN expression was statistically higher in T2DM patients than in healthy individuals ( $p < 0.0001$ ) and T2DM patients with cancer ( $p < 0.0001$ ). Furthermore, a statistically significant difference was observed between T2DM+C patients and healthy individuals ( $p = 0.0045$ ). HI: Healthy individuals, T2DM: patients with Diabetes Mellitus Type 2, T2DM+C: patients with Type 2 Diabetes Mellitus and cancer.

**Figure 3: Frequency of cases with PTEN expression >17.43.** Percentage calculated taking into account the cut-off value obtained from HI ( $\bar{x} + 3SD = 17.43$ ). High expression levels of *PTEN* among T2DM compared with T2DM+C and HI. Dark gray column: PTEN expression >17.43. Gray column: PTEN expression <17.43. HI: Healthy individuals, T2DM: patients with Type 2 Diabetes Mellitus, T2DM+C: patients with Type 2 Diabetes Mellitus and cancer.

Figure 1

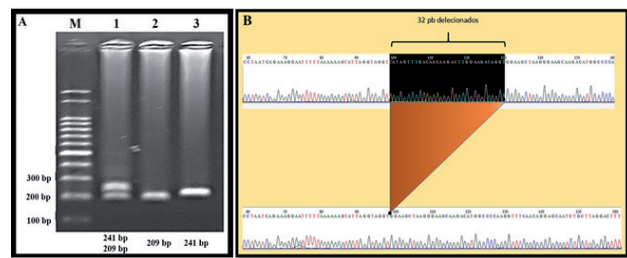


Figure 2

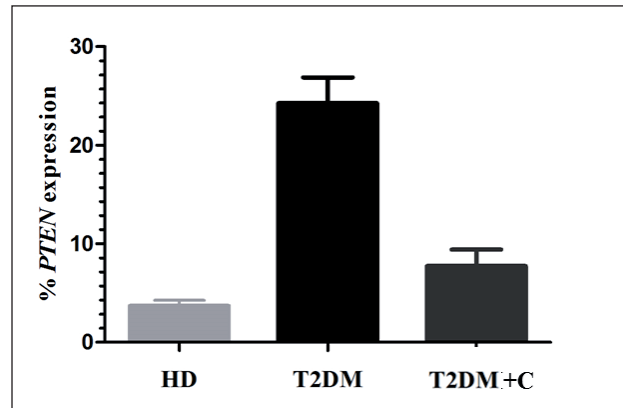


Figure 3

