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The Pro12Ala *PPARg2* gene polymorphism involves residual C-peptide secretion and BMI in type 1 diabetes

Polimorfizm Pro12Ala genu *PPARg2* jest związany z resztkową insulinosekrecją oraz wartością BMI w cukrzycy typu 1

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Abstract

Introduction and aim. Since insulin resistance is genetically determined and observed in type 1 diabetes, the study was designed to elucidate an involvement of Ala^{12}Pro *PPARg2* gene polymorphism in residual C-peptide secretion and BMI variation in children with type 1 diabetes. **Material and methods.** In 103 patients with type 1 diabetes genetic analysis of *PPARg2* polymorphism, C-peptide measurements and evaluation of BMI and clinical parameters were performed. Control group consisted of 109 healthy subjects. **Results.** In diabetic patients, only three individuals exhibited Ala^{12}Ala genotype (2.9%) and 29 patients were heterozygous Ala^{12}Pro (28.2%). Interestingly, Ala12+ variants were associated with higher C-peptide levels in 6th, 12th and 24th months after the onset than Pro^{12}Pro genotype (0.39±0.24 pmol/mL *vs*. 0.22±0.14 pmol/mL, *P*=0.007 and 0.19±0.09 *vs*. 0.11±0.07, *P*=0.01 and 0.13±0.09 *vs*. 0.07±0.05, *P*=0.021, respectively). Similarly, C-peptide was also significantly increased in patients with history of type 2 diabetes in the first-degree relatives. The observation was even more evident when Ala12+ variants were taken together with family history of type 2 diabetes. Besides, in 24th and 36th months after the onset, Ala12+ variants revealed to be associated with higher BMI normalized by age and sex as compared to Pro^{12}Pro (0.557±0.84 *vs*. -0.119±0.73, *P*=0.001 and 0.589±0.919 *vs*. 0.066±0.630, *P*=0.016, respectively). **Conclusions.** Thus, it is likely that *PPARg2* gene polymorphism and/or the genetically determined insulin resistance may be associated with residual C-peptide secretion and involve excessive BMI in type 1 diabetes.

Key words

PPAR receptors, genetics, C-peptide, body mass index

Streszczenie

Wprowadzenie i cel pracy. Z uwagi na występowanie w cukrzycy typu 1 (T1D) zjawiska insulinooporności oraz możliwość jej genetycznego uwarunkowania celem pracy była ocena wpływu polimorfizmu Ala^{12}Pro genu *PPARg2* na resztkową insulinosekrecję mierzoną stężeniem peptydu C oraz zmiany wartości indeks BMI u dzieci z cukrzycą typu 1. **Materiał i metody.** W grupie 103 pacjentów z T1D została przeprowadzona analiza genetyczna polimorfizmu genu *PPARg2*, pomiary stężenia peptydu C oraz ocena indeksu BMI i parametrów klinicznych. Grupa kontrolna obejmowała 109 zdrowe osoby. **Wyniki.** U pacjentów z T1D jedynie 3 dzieci posiadało genotyp Ala^{12}Ala (2.9%), podczas gdy 29 pacjentów było heterozygotami Ala^{12}Pro (28.2%). Interesujące, że warianty Ala12+ były związane z wyższym stężeniem peptydu C w 6, 12 oraz 24 miesiącu trwania choroby w porównaniu z genotypem Pro^{12}Pro (0.39±0.24 pmol/mL *vs*. 0.22±0.14 pmol/mL, *P*=0.007 i 0.19±0.09 *vs*. 0.11±0.07, *P*=0.01 oraz 0.13±0.09 *vs*. 0.07±0.05, *P*=0.021, odpowiednio). Podobnie stężenie peptydu C było istotnie wyższe u pacjentów z dodatnim wywiadem w kierunku cukrzycy typu 2 u krewnych pierwszego stopnia. Obserwacja ta była jeszcze bardziej wyraźna, gdy warianty Ala12 + były oceniane łącznie z rodzinnym wywiadem cukrzycy typu 2. Ponadto pacjenci, u których stwierdzono warianty Ala12+ charakteryzowali się w 24 i 36 miesiącu trwania cukrzycy wyższym indeksem BMI znormalizowanym pod względem płci i wieku w porównaniu do nosicieli Pro^{12}Pro (0.557±0.84 *vs*. -0.119±0.73, *P*=0.001 i 0.589±0.919 *vs*. 0.066±0.630, *P*=0.016, odpowiednio). **Wnioski.** Wydaje się prawdopodobne, że polimorfizm genu *PPARg2* i/lub genetycznie uwarunkowana insulinooporność mogą być związane z resztkową insulinosekrecją oraz wzrostem BMI w cukrzycy typu 1 u dzieci.

Słowa kluczowe

receptory PPAR, genetyka, peptyd C, indeks masy ciała

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Introduction

Natural course of type 1 diabetes could be demonstrated as a progressive decline in endogenous insulin secretion capacity caused by autoimmune destruction of the pancreatic β cells. The loss of immunological tolerance towards the pancreatic β cells initiates damage of insulin producing cells, which involves both the genetic and environmental factors and leads to the total insulin deficiency. However, the clinical symptoms of insulin insufficiency appear before all pancreatic β cells are damaged [1–3]. Preservation of the remaining β cell mass in patients at or prior to the onset of clinical disease is an important goal in improving prognosis since C-peptide is thought to have a beneficial effect on some clinical parameters, e.g. diabetic patients with residual b cell function are less prone to develop the diabetic micro- and macroangiopathy [4,5]. The residual β cell function could be studied by fasting or stimulated C-peptide serum level, which is secreted equimolar amounts with insulin after cleavage of proinsulin, and accurately reflects insulin secretion [6,7]. It is likely the variability of C-peptide secretion in type 1 diabetes may be involved by immunological β cell destruction, toxicity of hyperglycemia, modulation of insulin expression within b cells as well as the peripheral insulin resistance. There is some evidence that C-peptide level at type 1 diabetes onset as well as insulin resistance in healthy children are age-dependent features and, therefore, they may involve each other [8–11]. The insulin resistance, which is a typical feature of type 2 diabetes, very often coexists with obesity [12,13]. Moreover, plethora of the published data suggest insulin resistance to be genetically determined [12,14]. The one of the inherited factors associated with insulin resistance and obesity is a gene encoding peroxisome proliferator activated receptor-gamma (PPARg), which is a nuclear receptor for insulin sensitivity, lipids metabolism and adipocyte differentiation [15–17]. Two isoform molecules: PPARg1 and PPARg2, are constituted by use of alternative promoters and differential splicing of the same gene. Human PPARg2, which has additional 28 amino acids at N-terminus compared to PPARg1, is expressed almost exclusively in adipocyte tissue. There is also some evidence that PPARg action is ligand dependent, e.g. fatty acids and prostaglandin derivatives. Moreover, thiazolidinediones, which enhance sensitivity to insulin *in vitro* and *in vivo*, also stimulate PPARg receptors [18]. Thus, *PPARg2* gene is a putative candidate gene for several human disorders including type 2 diabetes, insulin resistance and obesity. A genetic variant of *PPARg2* gene (at nucleotide 34 C®G) has been reported [19]. It predicts substitution of alanine for the proline at amino acid 12 and may involve protein structure and function since it is non-conservative alteration. Moreover, reproducible data assume association of this polymorphism within *PPARg2* gene and insulin resistance as well as obesity in some populations [15,18,20]. To date Ala^{12}Pro of *PPARg2* gene polymorphism was not confirmed to involve insulin resistance in Polish Caucasians with type 2 diabetes [21].

Aim

In the light of the facts presented above, the study aimed to elucidate the association of *PPARg2* gene variants and family history of type 2 diabetes with residual C-peptide secretion and body weight in children with type 1 diabetes.

Material and methods

The investigation was in accordance with the principles of the Declaration of Helsinki, and the appropriate ethical committee approved the work. Informed written consent was obtained from all subjects. Patients with type 1 diabetes (T1D) consisting of 61 males and 42 females (mean age at the onset 9.6±4.0 years) were recruited from a group of diabetic children, which previously served for class 2 HLA genotyping [22]. Healthy 68 males and 41 females (mean age 10.4±3.4) were subjected as controls subjects. Both groups were Caucasians from the population in Central Poland. History of type 2 diabetes in the first degree relatives was clinically confirmed in 41 of 103 patients and 32 of 109 healthy individuals. Mean age at diagnosis of type 2 diabetes in relatives was 48.3±12.7 years and 8 of 41 individuals were treated with insulin. No fully confirmed BMI data for these subjects were available.

Both groups were applied for genetic analysis and only children with diabetes were enrolled to other evaluations. T1D individuals were positive for at least one of islet antibody e.g. 41 (39.8%) were ICA+, antiGAD+, IA2+; 23 (22.3%) ICA+, antiGAD+; 21 (20.4%) ICA+, IA2+; 10 (9.7%) only ICA+; 7 (6.8%) only antiGAD. One subject was negative for all islet antibodies, however, he has diabetic ketoacidosis, predisposing *HLA-DQA1*0301-DQB1*0302* haplotype and low C-peptide at the onset (0.141 pmol/mL). All the patients were dependent on insulin from the time of diagnosis. Approximately, 22% of the children exhibited diabetic ketoacidosis and the remaining subjects had hyperglycaemia combined with ketonuria (mean glycemia 23.4±11.2 mmol/dL). All the children were treated during the first 72 h by intravenous human regular insulin infusion. They continued to receive subcutaneous insulin therapy with regular rapid-acting and intermediate-acting insulin in 2-3 daily doses (15 of 103) or in 4-5 daily doses (88 of 103). All the patients were educated to self-monitor blood and urine for glucose, changing insulin doses to maintain preprandial glycaemia between 3.9-6.7 mmol/dL and postprandial blood glucose level between 5.6-8.9 mmol/dL. Follow-up visits were performed at day of diagnosis (1st day) and at 6th, 12th, 24th, 36th month of disease duration for all patients. However, in the 36th month only 68 patients were studied because some children were lost from the observation or not all data were available.

Genetic analysis of PPARg2 polymorphism. DNA was isolated in presence of proteinase K followed by a conventional salting-out procedure from 1 mL of EDTA freshly withdrawn blood. The target region of *PPARg2* gene was amplified in the presence of the following sense 5'-GCCAATTCAAGCCCAGTC-3' and antisense 5'-GATATGTTTGCAGACAGTGT

ATCAGTGAAGG-3' primers. The PCR cycling profile was as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 30 s, with final extension at 72 °C for 7 min. PCR product was 258 bp in length. For identifying C to G transversion (Pro^{12}Ala) PCR products were digested with *Bsh*1236I (MBI Fermentas, Vilnius, Lithuania) restriction enzyme (120 min at 37 °C, which gave shortened product of 215 bp if mutation was present. DNA fragments were identified in 3% agarose gel electrophoresis after staining with ethidium bromide.

C-peptide measurement. C-peptide serum levels were estimated applying the radioimmunoassay kit C-PEP-CT2 (CIS Bio International, France). All samples were measured in duplicate. Fasting C-peptide levels from healthy individuals ranged from 0.28 to 1.32 pmol/mL and the detection limit has been assessed as 0.025 pmol/mL for the assay.

Blood glucose, insulin requirement and HbA_{1c}. Fasting blood glucose was determined by the glucose oxydase method. Insulin requirement was assessed as a mean value of insulin units per kilogram body mass per day (IU/kg/24 h) after three days of continuous intravenous insulin infusion at the onset of type 1 diabetes and as a mean value of insulin units per kilogram per day calculated from three days of subcutaneous injections preceding each follow-up. HbA_{1c} was determined by high-performance liquid chromatography method (HPLC) (Bio-Rad, Munich, Germany).

Body mass index. BMI was calculated according to the following formula: BMI = weight (kilograms)/height^2 (meters) and was normalized for age and sex by converting it to Z-score (the number of SD above or below the mean for age and sex). Normal age related BMI data established in 6082 children (3005 males and 3077 females, age range 1-18 years), obtained from Central Poland register, were used for Z-score calculation [23].

Islets antibodies measurements and HLA-DQA1, DQB1 and DRB1 genes typing. All these measurements were performed as previously described [22]. Briefly, ICA were determined by indirect immunofluorescence method, antiGAD and IA2 antibodies were measured by means of the radio-immunological method with human recombinant antigens. HLA class II alleles were identified by PCR following hybridization with sequence specific oligonucleotide probes (SSOP) according to the XIIth International Histocompatibility Workshop protocols.

Statistical analysis. The significance of differences in alleles and genotypes between diabetic patients and controls was estimated by the chi-square (*chi*^{2}) test with Yates's correction or Fisher's exact test. Additionally, when $P<0.05$ odd ratio (*OR*) with 95% confidence interval (*95%CI*) was calculated. In order to check how normal the distribution of variables was, we used W Shapiro-Wilks' test, which assesses departures from normality. Significance of differences was estimated using Student t-test or Mann-Whitney's U test depending on the normality of data set for independent samples. Comparison between each follow-up data was performed using the Student t-test or Wilcoxon's signed rank test for dependent samples. For more detailed stratification, the multivariate analysis of variance (MANOVA) was applied after transformations to log base 10 to give more normal distribution. In that case Tukey's honest significant differences test for multiple comparisons was used. Correlation between parametric variables was assessed using Spearman's rank correlation test.

Results

Applying polymerase chain reaction followed by restriction fragments length polymorphism (*Bsh*1236I), we found the frequencies of Pro^{12}Ala *PPARg2* gene polymorphism were similar in both diabetic patients (Ala^{12} allele frequency 0.17) and healthy controls (Ala^{12} 0.15) of Polish origin. Genotypes distribution was also similar and conformed to expectations of the Hardy-Weinberg rule. In diabetic patients group, only three individuals exhibited Ala^{12}Ala genotype (2.9%) and 29 patients were heterozygous Ala^{12}Pro (28.2%). Therefore, because of a small number of Ala^{12}Ala homozygotes, these were collapsed with heterozygotes (Ala^{12}+), and compared with Pro^{12}Pro homozygotes for further analysis (Table 1).

37 (36%) of our studied patients have C-peptide in normal range. However, C-peptide level decreased significantly after two years of disease duration compared to the day of diagnosis (0.22±0.20 pmol/mL *vs.* 0.093±0.075 pmol/mL, $P<10^{-5}$). No linear correlation with diabetes duration was found (R=-0.93, P=0.066). After three years of diabetes duration only 9 of 68 remaining patients have C-peptide above detection limit of the assay applied. In these 9 individuals with persisting C-peptide level at the 3rd year after the onset, C-peptide level decline also differed significantly starting at the 6th after the onset (Table 2). Interestingly, these 9 individuals varied dramatically in terms of Ala allele frequency as compared to other patients (0.39 *vs*. 0.13, P=0.014, OR(95%CI)=4.37(3.89-4.84).

However, positive correlations of C-peptide level at diagnosis, after 6 and 12 months with age at the onset were observed (R=0.46, P=0.008; R=0.68, P=0.00001 and R=0.49, P=0.004, respectively). A linear correlation between HbA_{1c} and C-peptide was also found at the onset (R=0.045, P=0.00004). Concerning the polymorphism of *PPARg2* gene, Ala^{12}+ variants were found associated with higher C-peptide levels in the 6th, 12th and 24th month after the onset as compared to Pro^{12}Pro genotype (0.39±0.24 pmol/mL *vs*. 0.22±0.14 pmol/mL, P=0.007 and 0.19±0.09 *vs*. 0.11±0.07, P=0.01 and 0.13±0.09 *vs*. 0.07±0.05, P=0.021, respectively). That was also confirmed in MANOVA after transformation to log base 10 to give more normal distribution (Table 1).

Higher C-peptide level in the 6th and in the 12th month after diagnosis was also observed in patients with the family history of type 2 diabetes (0.34±0.22 vs. 0.20±0.13; P=0.021 and 0.17±0.09 vs. 0.10± 0.07; P=0.011, respectively). Moreover, considering the family history of type 2 diabetes together with Pro^{12}Ala *PPARg2* gene polymorphism in MANOVA, the Ala^{12}+ group of individuals exhibited the further increase of C-peptide serum level as compared with other groups (0.44±0.23, P=0.013 and 0.22±0.08, P=0.015 for the 6th and 12th month,

Table I. Studied parameters characteristics by Pro[12]Ala *PPARg2* gene polymorphism
Tabela I. *Badane parametry zależnie od polimorfizmu genu Pro12Ala PPARg2*

Time after diagnosis	Variable	PPARg2 phenotype		*P* value
		Pro/Pro *n*=71	Pro/Ala+Ala/Ala *n*=32	
	Male/Female	44/27	17/15	-
	Age [years]	9.57±3.87	9.06±3.54	0.56
1st day	Log C-peptide*	-0.671±0.388	-0.621±0.306	0.42
	HbA_{1c} [%]	11.24±2.99	10.71±3.36	0.54
	Insulin [IU/kg/24 h]	1.02±0.49	0.96±0.57	0.76
	BMI [kg/m^2]	16.90±3.01	16.51±2.30	0.58
	Z score for BMI	-0.421±1.080	-0.250±0.993	0.52
6th month	Log C-peptide*	-0.758±0.347	-0.504±0.361	0.038
	HbA_{1c} [%]	7.18±1.30	7.22±1.16	0.90
	Insulin [IU/kg/24 h]	0.54±0.26	0.53±0.29	0.89
	BMI [kg/m^2]	17.56±2.64	17.38±1.97	0.58
	Z score for BMI	-0.133±0.781	0.051±0.714	0.33
12th month	Log C-peptide	-1.044±0.331	-0.757±0.227	0.016
	HbA_{1c} [%]	7.75±2.04	7.63±0.8	0.81
	Insulin [IU/kg/24 h]	0.64±0.28	0.67±0.35	0.66
	BMI [kg/m^2]	18.41±3.09	18.04±2.23	0.58
	Z score for BMI	0.073±0.901	0.231±0.972	0.48
24th month	Log C-peptide*	-1.286±0.403	-1.000±0.353	0.049
	HbA_{1c} [%]	8.76±1.71	8.24±2.50	0.24
	Insulin [IU/kg/24 h]	0.89±0.44	0.77±033	0.26
	BMI [kg/m^2]	18.24±2.73	18.79±4.36	0.58
	Z score for BMI	-0.119±0.73	0.557±0.84	0.001

Data presented as means ± SD
* fasting C-peptide levels [pmol/mL] transformed to log base 10 for more normal distribution

respectively; Figure 1). The similar observation was not confirmed for the 24th month, however, the C-peptide level significantly decreased in that time.

On the other hand, a linear correlation between duration of the clinical phase of the disease and BMI (R=0.98, $P<10^{-5}$) and normalized by age and sex BMI (Z-score) was found (R=0.96, P=0.03). However, no correlation of age at the onset and BMI and Z-score was shown. Furthermore, linear correlation between C-peptide level and BMI were at the 1st day and the 6th and 12th month after the onset (R=0.41 and P=0.0002; R=0.53 and P=0.0004; R=0.46 and P=0.004; respectively). Ala[12]+ variants were related to higher Z-score for BMI in 24th and the 36th month after diagnosis as compared to Pro[12]Pro genotype (0.557±0.84 *vs*. -0.119±0.73, P=0.001 and 0.589±0.919 *vs*. 0.066±0.630, P=0.016, respectively). A linear positive correlation of Z-score with diabetes duration was found for Ala[12]+ group (R=0.95, P=0.015).

Table II. Studied parameters characteristics by above and below detection limit for C-peptide (0.025 pmol/mL) at 36th month
Tabela II. *Badane parametry zależne od wartości c-peptydu poniżej i powyżej granicy wykrywalności dla c-peptydu (0.025 pmol/mL) w 36. miesiącu*

	C-peptide at 36th month			
	Variable	>0.025 pmol/mL *n*=9	<0.025 pmol/mL *n*=59	*P* value
	Male/Female	5/4	36/23	-
	Age at onset [years]	11.89±2.07	9.43±4.13	0.56
PPARg2 genotype/allele	Pro/Pro	3	46	0.036†
	Pro/Ala	5	11	
	Ala/Ala	1	2	
	Ala frequency	0.39 (7/18)	0.13(15/118)	0.014‡
1st day	Log C-peptide*	-0.565±0.293	-0.667±0.358	0.42
	HbA_{1c} [%]	11.24±2.99	10.71±3.36	0.54
	Insulin [IU/kg/24 h]	1.02±0.49	0.96±0.57	0.76
	BMI [kg/m^2]	16.90±3.01	16.51±2.30	0.58
	Z score for BMI	-0.421±1.080	-0.250±0.993	0.52
6th month	Log C-peptide*	-0.453±0.109	-0.727±0.385	0.09
	HbA_{1c} [%]	7.18±1.30	7.22±1.16	0.90
	Insulin [IU/kg/24 h]	0.54±0.26	0.53±0.29	0.89
	BMI [kg/m^2]	17.56±2.64	17.38±1.97	0.58
	Z score for BMI	-0.133±0.781	0.051±0.714	0.33
12th month	Log C-peptide	-0.660±0.148	-1.072±0.344	0.004
	HbA_{1c} [%]	7.75±2.04	7.63±0.8	0.81
	Insulin [IU/kg/24 h]	0.64±0.28	0.67±0.35	0.66
	BMI [kg/m^2]	18.41±3.09	18.04±2.23	0.58
	Z score for BMI	0.073±0.901	0.231±0.972	0.48
24th month	Log C-peptide*	-0.713±0.125	-1.326±0.335	$<10^{-5}$
	HbA_{1c} [%]	8.76±1.71	8.24±2.50	0.24
	Insulin [IU/kg/24 h]	0.89±0.44	0.77±033	0.26
	BMI [kg/m^2]	18.24±2.73	18.79±4.36	0.58
	Z score for BMI	-0.119±0.73	0.557±0.84	0.001

Data presented as means ± SD
* fasting C-peptide levels [pmol/mL] transformed to log base 10 for more normal distribution
† chi^2 statistics with Yates' correction for 2 df and OR(95%CI) for Ala/Pro – 5.45(4.82-6.09) and for Ala/Ala – 3.46(2.47-4.65)
‡ chi^2 statistics with Yates' correction for 1 df and OR(95%CI) for Ala allele – 4.37(3.89-4.84)

Discussion

Clinical onset of type 1 diabetes is related to β cells destruction and, in consequence, the lack of endogenous insulin. Inconsistently, 36% of our studied patients with clinical features of insulin deficiency have C-peptide in normal range and the similar observations were reported by others [1,3,24]. Higher C-peptide levels in older healthy children were also reported, it suggests fasting insulin secretion may be age-dependent [1,25]. Moreover, the insulin resistance in healthy pediatric individuals is also related to age [25]. Hence, it is likely that the insulin resistance may be an additional factor triggering clinical manifestation of type 1 diabetes. In insulin treated patients, an inverse relationship exists between glycemic control and insulin sensitivity [12,26]. Furthermore, insulin resistance in type 1 diabetes may be predicted by the application of clinical components, e.g. HbA_{1c}, hypertension and body weight parameters [27,28]. In our study, linear correlation between HbA_{1c} and C-peptide was found at the onset and it could result from chronic hyperglycemia, which induced insulin resistance [12]. In subsequent follow-ups, when better glycemic control was related to exogenous insulin, this observation was not confirmed.

The insulin resistance is also associated with obesity, hence parameters describing body weight were included into further analysis. Significantly low BMI was observed at the onset of type 1 diabetes, which is likely the result of dehydration and catabolic processes during insulin deficiency. Similar to others, further follow-ups revealed the linear correlation between duration of diabetes and BMI index [29].

The literature data suggest the Pro^{12}Ala *PPARg2* gene polymorphism is associated with excessive weight [30], concerning also pediatric individuals [31]. In our evaluations, Ala^{12}+ variants appeared to be associated with higher Z-score for BMI in the 24th and 36th month after diagnosis as compared to Pro^{12}Pro genotype. It is interesting that linear positive correlation of Z-score with diabetes duration was found only for the Ala^{12}+ group. It is in concordance with the findings of Witchel *et al*. who reported Pro^{12}Ala *PPARg2* gene polymorphism as a genetic marker indicating risk for obesity persisting into adolescence [31]. Family history of type 2 diabetes did not affect the BMI and Z-score in our study.

Summarizing, our data suggest fasting C-peptide level association with age at the onset of type 1 diabetes, hyperglycemia duration before diagnosis and *PPARg2* gene polymorphism. All these features are typical factors contributed to insulin resistance. Thus, it is likely that the genetically determined insulin resistance may be associated with earlier clinical manifestation of type 1 diabetes and, in consequence, could indirectly affects residual b cells secretion. Moreover, we found the increase in body mass index, which is also related to insulin resistance, was especially excessive in some individuals carrying Pro^{12}Ala *PPARg2* gene polymorphism.

On the other hand, Pro^{12}Ala *PPARg2* gene polymorphism involvement may be so delicate that it does not appear in association with insulin replacement requirements and improved HbA1c levels in children with type 1 diabetes.

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