Nabil KM, et al., J Ophthalmic Clin Res 2016, 3: 019
DOI: 10.24966/OCR-8887/100019

HSOA Journal of Ophthalmology and Clinical Research

Research Article

Association of the PPARγ2 Pro12Ala and C1431T Polymorphisms with Type 2 Diabetes and Diabetic Retinopathy in a Sample of Egyptian Patients

Karim Mahmoud Nabil[1], Lubna Mohamed Ibrahim Desouky[2]* and Ebtesam Mohamed Abdalla[2]

[1]*Department of Ophthalmology, Alexandria University, Egypt*

[2]*Department of Human Genetics, Alexandria University, Egypt*

Abstract

Background

Peroxisome Proliferator Activated Receptor Gamma (PPAR-γ) is a pleotropic transcription factor. Given the pivotal roles of PPARγ in regulating metabolism, several studies in various ethnic populations have explored the association between variants in this gene and susceptibility to diabetes and its complications with conflicting results.

Objective

To evaluate the association of Pro12Ala and C1431T polymorphisms of PPARγ2 gene with Type 2 Diabetes (T2D), the risk of developing Diabetic Retinopathy (DR) and the severity of retinopathy, in a sample of Egyptian patients.

Methods

We recruited a total of 60 T2D patients from those attending the Ophthalmology Department, Alexandria University main Hospital - Egypt; 30 with DR (18 non-proliferative and 12 proliferative DR) and 30 patients with T2D of > 10 years' duration who showed no signs of DR. A third group of 120 non-diabetic volunteers were included as controls. Polymerase Chain Reaction followed by restriction enzyme digestion (PCR-RFLP) was used to determine the Pro12Ala and C1431T variants of PPARγ2 in patients and control subjects. Genotype and allele frequency distributions among the different groups were compared using the chi-square test.

Results

The frequency of the T allele of C1431T variant was significantly higher in T2D subjects than in controls (X^2=8.5, p=0.004), whereas Pro12Ala was not significantly associated with T2D. Among diabetic subjects, no significant association was observed between these polymorphisms and DR or the severity of retinopathy.

Conclusion

Our findings support a role for the PPAR-γ2 C1431T, but not Pro12Ala, in the etiology of T2D, while they confirm lack of association between both polymorphisms and the risk to develop DR.

Keywords: Diabetes; Diabetic retinopathy; Egypt; C1431T; PPARγ2 gene; Pro12Ala

***Corresponding author:** Lubna Mohamed Ibrahim Desouky, Department of Human Genetics, Alexandria University, El-Horreya Avenue El- Hadra, Alexandria, Egypt, Tel: +20 34285455; +20 34282373; +20 34288233; Fax: +20 34283719; E-mail: dr_lubna_mohamed@yahoo.com

Citation: Nabil KM, Desouky LMI, Abdalla EM (2016) Association of the PPARγ2 Pro12Ala and C1431T Polymorphisms with Type 2 Diabetes and Diabetic Retinopathy in a Sample of Egyptian Patients. J Ophthalmic Clin Res 3: 019.

Received: December 15, 2015; **Accepted:** February 16, 2016; **Published:** March 01, 2016

Introduction

Diabetes mellitus is a chronic metabolic disease that became a major public health problem in both developed and developing countries [1]. The prevalence of Type 2 DM (T2D), which is the most common type of diabetes, has raised markedly with the increased prevalence of obesity and sedentary life style [2]. The main cause of T2D is unclear, accumulating evidences suggest a polygenic nature of the disease marked by the interaction of environmental and genetic factors [3]. The long-term diabetic vascular complications are considered the leading cause of morbidity and mortality in diabetic patients [1]. Diabetic Retinopathy (DR) is one of the most common microvascular complications of diabetes [4], and one of the leading causes of blindness worldwide [5].

Among an array of candidate genes selected from the proposed pathogenic pathways, Peroxisome Proliferator-Activated Receptors (PPARs) have been widely studied to verify their role in diabetes and diabetic complications. PPARs are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes controlling a variety of biological functions such as cellular differentiation and metabolism [6]. Three types of PPARs have been identified: alpha, gamma, and beta [7]. Alpha (α) is expressed in liver, kidney, heart, muscle and adipose tissue. Beta (β) is expressed markedly in the brain, adipose tissue, and skin. Gamma (γ) although transcribed by the same gene, this PPAR through alternative splicing is expressed in three forms: γ1 is expressed in virtually all tissues, including heart, muscle, colon, kidney, pancreas, and spleen, γ2 is expressed mainly in adipose tissue, while γ3 is expressed in macrophages, large intestine, and white adipose tissue [7,8].

PPAR-γ expression in the retina has a beneficial role in the modulation of inflammation, angiogenesis and apoptosis in retinal and endothelial cells, thereby ameliorating the retinal and endothelial damage caused by high glucose induced prolonged inflammation [9]. Moreover, PPAR-γ ligands are potent inhibitors of corneal angiogenesis and neo-vascularization [10,11].

A number of genetic variants in the PPAR-γ2 gene have been identified. Among these variants, the most prevalent is Pro12Ala missense mutation, which was first identified in 1997 [12]. Although the initial publication [13], reported a 75% risk reduction for

diabetes conferred by the Ala allele of this variant, the subsequent studies reported inconsistent findings [14-17]. The next most frequently occurring PPAR-γ2 polymorphism is a silent C to T substitution at nucleotide 1431 in the sixth exon, (Cytosine (C) 161 → Tyrosine (T) substitution which was identified by Meirhaegue et al., [18].

Among different populations, this polymorphism has been inconsistently associated with susceptibility to T2D and diabetic complications [19-21]. These conflicting reports encouraged us to investigate the impact of these two common PPARγ polymorphisms on the susceptibility to T2D and DR in a sample of Egyptian diabetic patients. Furthermore, to our knowledge, no association studies, evaluating the role of the C1431T variant in T2D, or investigating the associations of PPARγ2 variants with DR, have been published.

Materials and Methods

The study was conducted on 60 unrelated T2D patients selected from those attending the outpatient clinic of the Ophthalmology Department in Alexandria University Hospital, including 30 patients suffering from T2D proved to have Diabetic Retinopathy by fundus examination (DR group) and another 30 patients with T2D of more than 10 years' duration who showed no signs of DR (DWR group). One-hundred-twenty non-diabetic age- and sex-matched volunteers were recruited for participation as control subjects.

The study was conducted in accordance with the Declaration of Helsinki and an informed consent was obtained from all subjects after the purpose of the study was explained to them. After, medical and family histories were carefully taken; venous blood samples for the molecular analysis were collected in EDTA tubes. The molecular study was carried out in the molecular laboratory of the Human Genetics Department, Medical Research Institute, Alexandria University-Egypt.

Molecular Study

Genomic DNA was extracted from peripheral blood leukocytes by salting out technique [22].

The Pro12Ala SNP single nucleotide polymorphism in the in exon B of the PPAR-γ2 gene was investigated by Polymerase Chain Reaction (PCR) amplification of genomic DNA followed by Restriction-endonuclease digestion (RFLP); according to the method previously reported by Al-Shali et al., [23]. Amplification via Veriti Thermal Cycler (Applied Biosystems) was performed using the primers 5'-ACT CTG GGA GAT TCT CCT ATT GGC-3' (forward) and 5'-CTG GAA GAC AAA CTA CAA GAG-3' (reverse) (Invitrogen). The PCR reaction consisted of 30pg primers, 50ng of genomic DNA, 3mM $MgCl_2$, 200mM each dNTPS, 1 unit Taq (Thermoscientific). The PCR program was: 94°C for 4 min followed by 30 cycles of 30s at 94°C and 45s at 58°C and 72°C for 1 min, with a final extension step of 7 min at 72°C. The PCR products were digested with HaeIII fast digest restriction enzyme (Thermoscentific) according to the manufacturer's direction.

For the PPAR-γ2 c.1431C_T genotyping, the same method as above was used except for an annealing temperature of 60°C and the following primers: 5'- CTG AAT GTG AAG CCC ATT GAA -3' (forward) and 5'- GTG GCT CAG GAC TCT CTG CTA G -3' (reverse). The amplified segments were digested with Pml I fast digest restriction enzyme (thermo scientific) according to the manufacturer's instructions.

The digested PCR products were resolved by electrophoresis on 3% agarose gel stained with ethidium bromide for 20 minutes at 200V and were sized with reference to a 50-bp DNA ladder.

Statistical Analysis

Allele frequencies were estimated by direct gene counting. Chi square goodness-of-fit test was used to assess the Hardy-Weinberg equilibrium in the studied groups. Genotype and allele frequency distributions among the patients and controls were compared using chi-square test calculated to estimate the relative risk of T2D or DR.

Results

Demographic and clinical data

The T2D group included 40 females and 20 males; the male to female ratio was 1:2. Their ages ranged from 39-70 years with almost 80% of them in the age group 50-70 years with a mean and SD of 56.95±8.33. The duration of diabetes ranged from 10-46 years. Among the DR group there were 14 males and 16 females while 6 males and 24 females formed the DWR (diabetes without retinopathy) group. The age ranged from 39-70 years with a mean and SD of 57.35±8.88 among the diabetic patients with DR; while among the DWR group the age ranged from 42-70 years with a mean of 55.7±8.4 with no statistical significance.

The DR group included 18 patients suffering from Nonproliferative DR (NPDR group) and 12 patients with Proliferative DR (PDR group). In the NPDR group, there was 4 cases of severe NPDR, 4 of moderate NPDR and 10 of mild NPDR. Ophthalmologic examination revealed other ocular complications in the form of macular edema in 5 patients, vitreous hemorrhage in another 5 cases, preretinal hemorrhage, rubeosis iridis and central retinal vein occlusion each in one case.

Molecular data

The distribution of the PPAR-γ2 Pro12Ala genotypes and alleles in T2D patients and controls is shown in table 1. The observed genotype frequencies followed the Hardy-Weinberg equilibrium in both groups. The Pro allele was slightly higher among diabetic patients than controls; a difference that was not statistically different (X^2=0.001, p=0.9695). Similarly, the association between Pro12Ala genotypes and T2D was considered to be not statistically significant (X^2=0.740, p=0.691).

PPAR-γ2 Pro12Ala polymorphism		T2D patients (n=60)	Non-diabetic subjects (n=120)	X^2	P value
Genotype frequencies n (%)	Pro/Pro	26 (43.33)	47 (39.17)	0.740	0.691
	Pro/Ala	22 (36.67)	52 (43.33)		
	Ala/Ala	12 (20.00)	21 (17.50)		
Allele frequencies n (%)	Pro	74 (61.67)	146 (60.83)	0.001	0.9695
	Ala	46 (38.33)	94 (39.17)		

Table 1: The allele and genotype frequencies of PPAR-γ2 Pro12Ala among the T2D patients compared to control subjects.

Table 2, shows the distribution of the PPAR-γ2 C1431T genotypes and alleles in T2D patients and controls. The frequency of the observed genotypes, in both patients and controls, did not deviate from the Hardy-Weinberg expectations. Among the Egyptian subjects, T2D patients showed higher frequencies of the variant

genotypes (C/T and T/T) compared to their matched controls. Based on chi-square tests, this difference was statistically significant indicating a highly significant association between T2D with respect to C to T substitution in the nucleotide 1431 of PPAR-γ2 (X^2=10.4, p=0.006). Similar significant results were obtained when the statistical comparison was based on the observed allele numbers, instead of genotypes (X^2=8.5, p=0.004).

PPAR-γ2 C1431T polymorphisms		T2D patients (n=60)	Non-diabetic subjects (n=120)	X^2	P value
Genotype frequencies n (%)	C/C	8 (13.33)	42 (35.00)	10.4	0.006*
	C/T	36 (60.00)	60 (50.00)		
	T/T	16 (26.67)	18 (15.00)		
Allele frequencies n (%)	C	52 (43.33)	144 (60.00)	8.5	0.004*
	T	68 (56.67)	96 (40.00)		

Table 2: The allele and genotype frequencies of PPAR-γ2 C1431T among the T2D patients compared to control subjects.

In order to study a putative association of DR with PPAR-γ2 variants, the genotypes and alleles frequencies of each polymorphism were calculated for DR subgroup and compared to those of the DWR subgroup (Table 3). Based on chi-square tests, the distribution of neither the PPAR-γ2 Pro12Ala variant nor the PPAR-γ2 C1431T variant, showed a significant difference between the DR and DWR subgroup.

PPAR-γ2 gene		Pro12Ala polymorphism			C1431T polymorphism	
		DR (n=30)	DWR (n=30)		DR (n=30)	DWR (n=30)
Genotype frequencies n (%)	Pro/Pro	10 (33.34)	16 (53.33)	C/C	3 (10.00)	5 (16.67)
	Pro/Ala	13 (43.33)	9 (30.00)	C/T	21 (70.00)	15 (50.00)
	Ala/Ala	7 (23.33)	5 (16.67)	T/T	6 (20.00)	10 (33.33)
		X^2=2.45, p=0.294			X^2=2.50, p=0.287	
Allele frequencies n (%)	Pro	33 (55.00)	41 (68.33)	C	27 (45.00)	25 (41.67)
	Ala	27 (45.00)	19 (31.67)	T	33 (55.45)	35 (58.33)
		X^2=1.727, p=1.1887			X^2=0.34, p=0.8538	

Table 3: Distribution of the genotypes and alleles of PPAR-γ2 Pro12Ala and C1431T polymorphisms among the T2D patients according the presence of retinopathy.

In further, we explored the impact of the PPAR-γ2 variants on the severity of retinopathy. The DR patients were classified into subjects with NPDR and those with PDR, and then the genotype and allele frequencies of the studied polymorphism were calculated separately for each group as shown in table 4. When we compared the distribution of the genotypes and alleles of both polymorphism among the two groups, no statistically significant difference was found, indicating a lack of association between the PPAR-γ2 Pro12Ala or C1431T variants and the severity of DR.

Discussion

Among several genetic variants of the PPARγ2 gene, two polymorphisms; Pro12Ala of the exon B (rs1801282) and the C1431T silent substitution (rs3856806) in the exon 6, are the most frequently occurring SNPs and have been associated with various diseases [13-21]. The association of Pro12Ala polymorphism with T2D, insulin resistance, obesity and metabolic disorders has been reported in several studies [24-26]. The C1431T polymorphism has also been studied in relation to obesity, diabetes and coronary heart disease [18,25-27]. After a meticulous review of the literature, we were able to find a single association study conducted on Egyptian individuals to explore the relation between Pro12Ala PPARγ2 variant and T2D risk [28]. However, to our knowledge, no association studies, evaluating the role of the C1431T variant in T2D, or investigating the associations of PPARγ2 variants with DR, have been published.

PPAR-γ2 gene		Pro12Ala polymorphism			C1431T polymorphism	
		PDR (n=12)	PDR (n=12)		PDR (n=12)	NPDR (n=18)
Genotype frequencies n (%)	Pro/Pro	3 (25.00)	7 (38.89)	C/C	1 (08.33)	2 (11.11)
	Pro/Ala	7 (58.33)	6 (33.33)	C/T	8 (66.67)	13 (72.22)
	Ala/Ala	2 (16.67)	5 (27.78)	T/T	3 (25.00)	3 (16.67)
		X^2=1.84, p=0.399			X^2=0.337, p=0.845	
Allele frequencies n (%)	Pro	13 (54.17)	20 (55.56)	C	10 (41.67)	17 (47.22)
	Ala	11 (45.83)	16 (44.44)	T	14 (58.33)	19 (52.78)
		X^2=0.011, p=0.916			X^2=0.025, p=0.874	

Table 4: Distribution of the genotypes and alleles of PPAR-γ2 Pro12Ala and C1431T polymorphisms among patients with DR according the type of retinopathy.

In the present study, we investigated the impact of Pro12Ala and C1431T PPARγ2 polymorphisms on the risk to develop T2D and DR and we came out with two important findings. The first is the significantly higher risk for diabetes among carriers of the T allele of the C1431T polymorphism. The second finding is that the frequency of the Ala12 allele of PPARγ2 in the Egyptian sample studied was very high (~38% in T2D cases and ~39% in controls) compared to that reported for Caucasians [14,16,29,30]. However, the protective association of the "Ala" allele with T2D was not confirmed in the present work. In addition, our study failed to find any significant association of the two studied polymorphisms with the risk of DR among T2D patients.

The PPARγ2 Pro12Ala variant was not associated with either T2D or DR in the current study. In previous studies, the association between the Pro12Ala polymorphism and fasting glucose or insulin concentrations have yielded conflicting results [14,16,17,26]. Overall, combined evidences suggest that the Ala12 allele exerts a protective effect with carriers of this allele causing a decreased diabetes risk [13,15,17,24,26,31,32]. However, several reports from different ethnic origins failed to reproduce a significant association [16,19,23,33,34]. Nevertheless, some studies have even found an increased risk for T2D in subjects with the Ala12 variant [35,36]. The results of the previous Egyptian study supported an association between Pro allele of PPARγ2 gene and T2D [28]. It is noteworthy that the unexplained high frequencies of the Ala allele observed among the Egyptian control subjects, in the previous study (53%) [28] and also in the current work (~39%), exceed any frequency reported.

Regarding the relation between the PPARγ2 Pro12Ala variant and DR, few reports are available and results were also controversial. Our

findings are consistent with Petrovic et al., and Costa et al., [37,38], but contradict others who reported a protective role of the 12Ala polymorphism against proliferative DR in individuals with T2D [39,40].

In the present study, despite the increased frequency of the T allele of C1431T polymorphism and the strong association with T2D, we did not find any significant difference of this variant between the DR and DWR subgroups, indicating that it confers risk for the development T2D but not for the DR complications. The CT and TT genotype of C1431T were significantly present in diabetic patients as a whole, but no genotype was significantly higher in patients with DR. Instead, the T allele was slightly higher in the DWR subgroup. A contradicting finding was reported by Tai et al., [25], they described a reduced risk of diabetes in carriers of the T allele of the C1431T polymorphism. In fact, only few studies [18,20,26,41] have analyzed the effects of the C1431T polymorphism, and none of them found a significant association of this variant with glucose or lipid-related variables. These inconsistencies may be attributable to the fact that this polymorphism is silent at the amino acid level and is unlikely to have any direct mechanistic link with specific phenotypes. Instead, it might be in linkage disequilibrium with an unidentified functional variation either within or flanking the PPARγ2 gene or in another gene at this locus.

We ensued to compare our results regarding the distribution of the PPARγ2 polymorphisms with the available reports from different Arab populations. In agreement with our finding, Badii et al., [42] could not confirm the potential association between this polymorphism and T2D among Qataris subjects but they reported a low frequency of the Ala allele (5.5% for diabetic and 5.9% for non diabetics) as compared to ours and to the previous reports in various Caucasians. In Tunisia, several researchers examined the association of the genetic variation of the PPARγ2 with the susceptibility to T2D and concluded that the PPARγ2 is unlikely to be responsible for T2D in the Tunisian population [43-45].

These discrepancies between studies related to these polymorphisms may be partly explained by the presence of a gene-gene or gene-environment interaction. The controversial findings may also be related to information bias caused by the studies design and the selection criteria of patients. In addition, the prevalence of these polymorphisms; which varies greatly among populations, and may be very low in some of them, may contribute to the inconsistent results by affecting the statistical power of the comparisons.

In conclusion, the present study describes a significantly higher risk for diabetes among carriers of the T allele of the C1431T polymorphism, while it reports lack of association of the Pro12Ala variants with T2D. We also found no evidence of an association between the studied variants of PPARγ2 gene with DR in the T2D patients. Thus, the PPARγ2 C1431T variant seems to confer risk for the development of T2D but not for its chronic complications. Finally, since the lack of association might come from the small sample size, further studies on larger sample sizes are required to verify the present observation.

References

1. Deshpande AD, Harris-Hayes M, Schootman M (2008) Epidemiology of diabetes and diabetes-related complications. Phys Ther 88: 1254-1264.
2. Sullivan PW, Morrato EH, Ghushchyan V, Wyatt HR, Hill JO (2005) Obesity, inactivity, and the prevalence of diabetes and diabetes-related cardiovascular comorbidities in the US, 2000-2002. Diabetes Care 28: 1599-1603.
3. Groop LC (1997) The molecular genetics of non-insulin-dependent diabetes mellitus. J Intern Med 241: 95-101.
4. Fong DS, Aiello LP, Ferris FL 3rd, Klein R (2004) Diabetic retinopathy. Diabetes Care 27: 2540-2553.
5. Bourne RR, Jonas JB, Flaxman SR, Keeffe J, Leasher J, et al. (2014) Prevalence and causes of vision loss in high-income countries and in Eastern and Central Europe: 1990-2010. Br J Ophthalmol 98: 629-638.
6. Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 20: 649-688.
7. Berger J, Moller DE (2002) The mechanisms of action of PPARs. Annu Rev Med 53: 409-435.
8. Rosen ED, Spiegelman BM (2001) PPARgamma: a nuclear regulator of metabolism, differentiation, and cell growth. J Biol Chem 276: 37731-37734.
9. Xin X, Yang S, Kowalski J, Gerritsen ME (1999) Peroxisome proliferator-activated receptor gamma ligands are potent inhibitors of angiogenesis *in vitro* and *in vivo*. J Biol Chem 274: 9116-9121.
10. Muranaka K, Yanagi Y, Tamaki Y, Usui T, Kubota N, et al. (2006) Effects of peroxisome proliferator-activated receptor gamma and its ligand on blood-retinal barrier in a streptozotocin-induced diabetic model. Invest Ophthalmol Vis Sci 47: 4547-4552.
11. Murata T, He S, Hangai M, Ishibashi T, Xi XP, et al. (2000) Peroxisome proliferator-activated receptor-gamma ligands inhibit choroidal neovascularization. Invest Ophthalmol Vis Sci 41: 2309-2317.
12. Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, et al. (1997) Molecular scanning of the human Peroxisome Proliferator Activated Receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. Biochem Biophys Res Commun 241: 270-274.
13. Deeb SS, Fajas L, Nemoto M, Pihlajamäki J, Mykkänen L, et al. (1998) A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 20: 284-287.
14. Clement K, Hercberg S, Passinge B, Galan P, Varroud-Vial M, et al. (2000) The Pro115Gln and Pro12Ala PPAR gamma gene mutations in obesity and type 2 diabetes. Int J Obes Relat Metab Disord 24: 391-393.
15. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, et al. (2000) The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. Biochem Biophys Res Commun 271: 212-216.
16. Mancini FP, Vaccaro O, Sabatino L, Tufano A, Rivellese AA, et al. (1999) Pro-12Ala substitution in the peroxisome proliferator-activated receptor-gamma2 is not associated with type 2 diabetes. Diabetes 48: 1466-1468.
17. Ringel J, Engeli S, Distler A, Sharma AM (1999) Pro12Ala missense mutation of the peroxisome proliferator activated receptor gamma and diabetes mellitus. Biochem Biophys Res Commun 254: 450-453.
18. Meirhaeghe A, Fajas L, Helbecque N, Cottel D, Auwerx J, et al. (2000) Impact of the peroxisome proliferator activated receptor gamma2 Pro12Ala polymorphism on adiposity, lipids and non-insulin-dependent diabetes mellitus. Int J Obes Relat Metab Disord 24: 195-199.
19. Pattanayak AK, Bankura B, Balmiki N, Das TK, Chowdhury S, et al. (2014) Role of peroxisome proliferator-activated receptor gamma gene polymorphisms in type 2 diabetes mellitus patients of West Bengal, India. J Diabetes Investig 5: 188-191.
20. Poulsen P, Andersen G, Fenger M, Hansen T, Echwald SM, et al. (2003) Impact of two common polymorphisms in the PPARgamma gene on glucose tolerance and plasma insulin profiles in monozygotic and dizygotic twins: thrifty genotype, thrifty phenotype, or both? Diabetes 52: 194-198.
21. Tavares V, Hirata RD, Rodrigues AC, Monte O, Salles JE, et al. (2005) Effect of the peroxisome proliferator-activated receptor-gamma C161T polymorphism on lipid profile in Brazilian patients with Type 2 diabetes mellitus. J Endocrinol Invest 28: 129-36.

22. Salazar LA, Hirata MH, Cavalli SA, Machado MO, Hirata RD (1998) Optimized procedure for DNA isolation from fresh and cryopreserved clotted human blood useful in clinical molecular testing. Clin Chem 44: 1748-1750.

23. Al-Shali KZ, House AA, Hanley AJ, Khan HM, Harris SB, et al. (2004) Genetic variation in PPARG encoding peroxisome proliferator-activated receptor gamma associated with carotid atherosclerosis. Stroke 35: 2036-2040.

24. Stumvoll M, Häring H (2002) The peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism. Diabetes 51: 2341-2347.

25. Tai ES, Corella D, Deurenberg-Yap M, Adiconis X, Chew SK, et al. (2004) Differential effects of the C1431T and Pro12Ala PPARgamma gene variants on plasma lipids and diabetes risk in an Asian population. J Lipid Res 45: 674-685.

26. Vergotine Z, Yako YY, Kengne AP, Erasmus RT, Matsha TE (2014) Proliferator-activated receptor gamma Pro12Ala interacts with the insulin receptor substrate 1 Gly972Arg and increase the risk of insulin resistance and diabetes in the mixed ancestry population from South Africa. BMC Genetics 15: 10.

27. Blüher M, Klemm T, Gerike T, Krankenberg H, Schuler G, et al. (2002) Lack of association between peroxisome proliferator-activated receptor-gamma-2 gene variants and the occurrence of coronary heart disease in patients with diabetes mellitus. Eur J Endocrinol 146: 545-551.

28. Sokkar S, El-Sharnouby JA, Helmy A, El-Bendary A, Ahmad LS, et al. (2009) Role of Peroxisome Proliferator-Activated Receptor gamma2 (PPAR-γ2) gene polymorphism in type 2 diabetes mellitus. European Journal of General Medicine 6: 78-86.

29. Beamer BA, Yen CJ, Andersen RE, Muller D, Elahi D, et al. (1998) Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor-gamma2 gene with obesity in two Caucasian populations. Diabetes 47: 1806-1808.

30. Wu Z, Lou Y, Jin W, Liu Y, Lu L, et al. (2012) The Pro12Ala polymorphism in the Peroxisome Proliferator-Activated Receptor gamma-2 gene (PPARγ2) is associated with increased risk of coronary artery disease: a meta-analysis. PLoS One 7: 53105.

31. Mori H, Ikegami H, Kawaguchi Y, Seino S, Yokoi N, et al. (2001) The Pro12 -->Ala substitution in PPAR-gamma is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. Diabetes 50: 891-894.

32. Douglas JA, Erdos MR, Watanabe RM, Braun A, Johnston CL, et al. (2001) The peroxisome proliferator-activated receptor-gamma2 Pro12A1a variant: association with type 2 diabetes and trait differences. Diabetes 50: 886-890.

33. Evans D, de Heer J, Hagemann C, Wendt D, Wolf A, et al. (2001) Association between the P12A and c1431t polymorphisms in the Peroxisome Proliferator Activated Receptor gamma (PPAR gamma) gene and type 2 diabetes. Exp Clin Endocrinol Diabetes 109: 151-154.

34. Oh EY, Min KM, Chung JH, Min YK, Lee MS, et al. (2000) Significance of Pro12Ala mutation in peroxisome proliferator-activated receptor-gamma2 in Korean diabetic and obese subjects. J Clin Endocrinol Metab 85: 1801-1804.

35. Hegele RA, Cao H, Harris SB, Zinman B, Hanley AJ, et al. (2000) Peroxisome proliferator-activated receptor-gamma2 P12A and type 2 diabetes in Canadian Oji-Cree. J Clin Endocrinol Metab 85: 2014-2019.

36. Lindi VI, Uusitupa MI, Lindström J, Louheranta A, Eriksson JG, et al. (2002) Association of the Pro12Ala polymorphism in the PPAR-gamma2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. Diabetes 51: 2581-2586.

37. Costa V, Casamassimi A, Esposito K, Villani A, Capone M, et al. (2009) Characterization of a novel polymorphism in PPARG regulatory region associated with type 2 diabetes and diabetic retinopathy in Italy. J Biomed Biotechnol 2009: 126917.

38. Petrovic MG, Kunej T, Peterlin B, Dovc P, Petrovic D (2005) Gly482Ser polymorphism of the peroxisome proliferator-activated receptor-gamma coactivator-1 gene might be a risk factor for diabetic retinopathy in Slovene population (Caucasians) with type 2 diabetes and the Pro12Ala polymorphism of the PPARgamma gene is not. Diabetes Metab Res Rev 21: 470-474.

39. Malecki MT, Cyganek K, Mirkiewicz-Sieradzka B, Wolkow PP, Wanic K, et al. (2008) Alanine variant of the Pro12Ala polymorphism of the PPARgamma gene might be associated with decreased risk of diabetic retinopathy in type 2 diabetes. Diabetes Res Clin Pract 80: 139-145.

40. Tariq K, Malik SB, Ali SH, Maqsood SE, Azam A, et al. (2013) Association of Pro12Ala polymorphism in peroxisome proliferator activated receptor gamma with proliferative diabetic retinopathy. Mol Vis 19: 710-717.

41. Valve R, Sivenius K, Miettinen R, Pihlajamäki J, Rissanen A, et al. (1999) Two polymorphisms in the peroxisome proliferator-activated receptor-gamma gene are associated with severe overweight among obese women. J Clin Endocrinol Metab 84: 3708-3712.

42. Badii R, Bener A, Zirie M, Al-Rikabi A, Simsek M, et al. (2008) Lack of association between the Pro12Ala polymorphism of the PPAR-gamma 2 gene and type 2 diabetes mellitus in the Qatari consanguineous population. Acta Diabetol 45: 15-21.

43. Bouhaha R, Meyre D, Kamoun HA, Ennafaa H, Vaillant E, et al. (2008) Effect of ENPP1/PC-1-K121Q and PPARgamma-Pro12Ala polymorphisms on the genetic susceptibility to T2D in the Tunisian population. Diabetes Res Clin Pract 81: 278-283.

44. Mohamed MB, Mtiraoui N, Ezzidi I, Chaieb M, Mahjoub T, et al. (2007) Association of the peroxisome proliferator-activated receptor-gamma2 Pro12Ala but not the C1431T gene variants with lower body mass index in Type 2 diabetes. J Endocrinol Invest 30: 937-943.

45. Zouari Bouassida K, Chouchane L, Jellouli K, Chérif S, Haddad S, et al. (2005) The peroxisome proliferator activated receptorgamma2 (PPARgamma2) Pro12Ala variant: lack of association with type 2 diabetes in obese and non obese Tunisian patients. Diabetes Metab 31: 119-123.