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The PPAR γ (P12A) Locus-Associated Diabetes Risk is Modulated by Central Obesity in Punjabi Sikhs

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Abstract

Background: Several studies have reported a common proline-to-alanine substitution (P12A) in the peroxisome proliferator-activated receptor gamma (PPARγ) gene to be invariably associated with obesity, insulin resistance, type 2 diabetes mellitus (T2DM) and dyslipidemia. The purpose of this study was to determine whether the PPARγ gene (P12A) polymorphism contributes to susceptibility to T2DM and central obesity in Khatri Sikh community from India.

Methods: We studied 1711 subjects comprising 1186 individuals from 324 families and 525 unrelated non-diabetic controls. We tested the association between T2D and P12A polymorphism using logistic regression before and after adjusting for age, gender, and other covariates. We also examined the impact of these variants on obesity, glucose homeostasis and lipid traits using multiple linear regression analysis.

Results: Our findings could not confirm the association of PPARγ polymorphism with T2DM in this family-based sample. However, the comparison of unrelated controls with affected relatives (n=537) revealed a marginally significant association with this locus with T2DM (odds ratio (OR) 1.48, *P*=0.016). However, in the data stratified by traits related to abdominal obesity, there was a significant increase in T2DM risk in the individuals with waist circumference (>37 inches, OR 3.51, *P*=0.005) and waist to hip ratio (>0.95, OR 3.02, *P*= 0.003) in younger relatives.

Conclusions: PPARγ (P12A) locus appears to have profound influence in promoting insulin sensitivity through its interaction with central obesity particularly at younger age.

Introduction

India has the highest number of diabetics in the world and type 2 diabetes mellitus (T2DM) has become a major public health problem in urban and sub-urban areas [1]. Despite the absence of conventional risk factors such as high smoking, obese body mass index (BMI), and diet rich in meats, people from India, as well as from the entire Indian subcontinent have high prevalence of a characteristic metabolic syndrome. This includes elevated plasma triglycerides and small low-density lipoprotein (LDL) particle, reduced high-density lipoprotein cholesterol (HDL-C), early onset of insulin resistance, central obesity, and premature atherosclerosis [2-9]. Insulin resistance is predominantly associated with obesity, especially when obesity is centrally distributed and becomes a major risk factor for developing T2DM and cardiovascular disease (CVD) [10,11].

Obesity and its metabolic consequences such as T2DM and CVD have increased to epidemic proportions during the past two decades. This phenomenon could be attributed to the adoption of sedentary and western lifestyles, and enhanced intake of high-density caloric diets with little physical activity. The modern day lifestyles have increased the genetic susceptibility to generalized metabolic conditions when central obesity is observed [12-15]. In particular the interplay between genes and central obesity and the pathophysiology of T2DM and CVD is complex and thus, it is likely that a common set of genes with pleiotropic effects might influence obesity, T2DM and CVD.

A candidate gene with pleiotropic effects is the peroxisome proliferator-activated receptor gamma (PPARγ). It is a nuclear hormone receptor and is an important regulator of adepocyte differentiation [16,17]. The critical role of PPARγ in the development of adipose tissue in mammals was confirmed by the absence of adipose tissue in the PPARγ knockout murine embryos [18]. The human PPARγ gene maps to chromosome 3p24, and a common missense mutation in PPARγ (proline12 to alanine12 or P12A) has been implicated in increasing T2DM risk in several independent datasets including recent genome-wide association studies (GWAS) and is widely studied for its role in insulin resistance, central obesity and T2DM, and other related phenotypes [17,19-24]. Differential splicing of human messenger RNA generates two different isoforms: PPARγ-1 and PPARγ-2 that differ at their 5' ends [25,26]. PPARγ-1 is expressed in diverse tissues including adipose, skeletal muscle, heart, liver, and large intestine, while PPARγ-2 is exclusively expressed in adipose tissues [27,28]. In addition, PPARγ-2 is involved in insulin signaling, inflammation, obesity, and the development of T2DM [29]. The 'Ala12' allele with lower transcriptional activity has been associated with reduced risk of T2DM [29]. However, the common 'Pro12' protein (with greater *in vitro* activity than 'Ala12') has been associated with decreased insulin sensitivity, obesity and T2DM in many but not all studies [19,30,31]. We have previously reported significant association of the P12A (rs1801282) with T2DM in this population [32]. To further define the role of the PPARG locus in T2DM pathophysiology and to discover functional variant in this gene, we performed a comprehensive screening using 14 tagging single nucleotide polymorphisms (tagSNPs) from the PPARG locus in our case-control cohort of Khatri Sikhs [23]. With the exception of a strong association of P12A with T2DM

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effects of age, BMI, waist and or waist to hip ratio were included as covariates in the regression analyses.

Estimates of relative risk of T2DM and other traits between genotypes were determined using odds ratios (ORs) using the Mantel-Haenzel method. The logistic regression was used to estimate odds ratios for the PPARγ (Pro/Pro vs. Pro/Ala+ Ala/Ala) polymorphisms after adjusting for the effects of sex, age, BMI, WC and/or WHR. We also performed multiple linear regression analysis for studying the impact of PPARγ polymorphism on T2DM risk using obesity (BMI, WC and WHR) as a dependent quantitative trait (independently) in combined cases, as well as separately in probands and affected relatives, when comparing the effect with unrelated controls. The possible effects of age and sex were also included in each model. The impact of PPARγ on age-of onset was assessed using Kaplan-Meier survival statistics. All statistical analyses were carried out using SPSS for Windows statistical package 14.0.

Results

Baseline clinical information of the study subjects

About 75% of the SDS subjects used in this investigation were migrant from Pakistan (Rawalpindi and Peshawar areas), part of the united Punjab province before India-Pakistan separation. The current study includes a total of 1711 subjects (58% males). The baseline clinical attributes for the entire study subjects classified into four different groups (probands, affected relatives, unaffected relatives and unrelated controls) are presented in Table 1 and the mean values are separated by gender. Among cases, 75% maintained glycemic control by using oral agents (~13% of these taking insulin), and the remaining subjects by diet and exercise. Altogether, 24% of the T2DM patients were obese (BMI>30 kg/m2) and 42% were overweight (BMI>24.9). The mean BMI values did not vary significantly among affected and unaffected control subjects (both related and unrelated) (Table 1). Despite having comparable BMI, the female cases (probands and affected relatives) presented significant adiposity, as reflected by their significantly higher WC ($p<0.0001$), WHR ($p<0.0001$), and hip circumference ($p<0.008$) values compared to unrelated control subjects. Higher values for WC were also observed in male cases, the difference however was not significant. Patients also had a wide range of diabetes-related complications and these clinical details have been described elsewhere [37]. As shown in Figure 1, the unaffected relatives showed higher predisposition to insulin resistance than the unrelated controls, as indicated by higher HOMA IR values. Perhaps the higher prevalence of insulin resistance among unaffected relatives may explain the over-representation of Pro/Pro genotype among relatives, which may predispose the younger unaffected relatives to develop early T2DM.

Allele and genotype frequencies of PPARγ (p12a) polymorphism

The distribution of PPARγ (P12A) was assessed separately in cases (probands and affected relatives) and control groups (unaffected relatives and unrelated non-diabetic controls). As shown in Table 2, the affected relatives (n=537) were further stratified into youngest affected non-probands (n=205) (unrelated), and the oldest affected non-probands (n=233) (unrelated) by choosing one affected member per family for 'youngest' and 'oldest' group. The remaining affected relatives (n=99) were grouped to 3rd category named 'other affected relatives'. The homozygotes for the rare 'Ala12' allele were not observed in the entire family sample while one Ala/Ala genotype was observed in unrelated controls. The distribution of genotype and allele frequencies for the PPARγ (P12A) polymorphism was within Hardy-Weinberg expectations among unrelated controls as well as in the related controls. However, these frequencies were not within Hardy-Weinberg expectations in the combined family sample (n=1186). Apparently, the combined sample is not a random sample from population and thus violates one of the assumptions of Hardy-Weinberg equilibrium. The observed departure signifies over-representation of 'Pro12' allele among families which disappears when we analyzed-each 'unrelated family subgroup' separately. However, to rule out genotyping errors, we repeated 28% of our sample, but without a significant change in the results.

The frequency of 'Pro/Ala' genotypes was similar between cases (proband; 18% affected relative; 17%) and controls (unaffected relatives; 16%) in the family sample comprising 1186 subjects. Comparison of probands (n=353) or affected relatives (n=537) with unrelated controls (n=525) revealed significant association. As shown in Table 2, the frequency of 'Pro/Ala' genotypes was significantly higher in unrelated controls compared to probands (20.8% vs. 18%; $P<0.001$) affected relatives (20.8% vs.17%; $P<0.001$), and even with unaffected relatives

	Gender	Proband 223M/130F (353)*	Affected Relatives 330M/207F (537)	Unaffected Relatives 188M/108F (296)	Unrelated Controls 253M/272F (525)	p-value**
Age (yrs.)	M F	54.06 ± 10.60 55.50 ± 9.86	51.69 ± 11. 87 56.06 ± 11.62	45.17 ± 14.88 50.11 ± 13.84	49.17 ± 15.12 48.98 ± 13.05	0.000 0.000
Age at Diagnosis (yrs.)	M F	45.33 ± 10.19 47.60 ± 10.07	43.51 ± 11.27 49.03 ± 10.91	--	--	
Duration (yrs.)	M F	8.87 ± 7.27 7.75 ± 6.58	7.19 ± 7.58 6.71 ± 6.51	--	--	
BMI (kg/m^2)	M F	26.67 ± 4.10 28.65 ± 5.648	27.13 ± 4.58 28.38 ± 4.73	27.12 ± 4.66 27.64 ± 4.73	26.00 ± 4.86 26.24 ± 4.95	0.107 0.000
Waist circumference (WC) (inches)	M F	37.40 ± 4.01 36.70 ± 5.19	37.59± 4.30 36.46 ± 3.80	36.86 ± 4.56 34.74± 4.05	36.39 ± 4.50 34.26 ± 4.48	0.011 0.000
Hip circumference (inches)	M F	37.70 ± 3.25 39.42 ± 4.85	37.78 ± 3.25 38.97 ± 3.94	37.95 ± 3.45 38.43 ± 3.84	37.63 ± 4.05 37.80 ± 3.89	0.836 0.008
WHR	M F	0.99 ± 0.06 0.93 ± 0.07	0.99 ± 0.07 0.94 ± 0.07	0.97 ± 0.07 0.90 ± 0.07	0.96 ± 0.07 0.91 ± 0.07	0.000 0.007
Sub-scapula (mm)	M F	28.78 ± 8.19 34.01 ± 8.08	29.39 ± 8.68 33.53 ± 7.69	28.82 ± 8.84 32.25 ± 8.38	27.12 ± 8.56 31.60 ± 9.34	0.032 0.012
Triceps(mm)	M F	17.88 ± 8.53 27.27 ± 8.31	18.33 ± 7.12 26.81 ± 8.02	18.12 ± 7.14 25.26 ± 7.65	16.59 ± 6.11 25.33 ± 7.85	0.056 0.024

Values are mean + SD; 'M' and 'F' indicate male and female;* figures in parentheses represent total subjects under each category; ** Difference between probands and unrelated controls

Table 1: Characteristics of Study Population.

	Total subjects (1711)	Mean Age (Affected relatives)	Pro /Pro Count (%)	Pro/Ala Count (%)	Ala/Ala Count (%)	Allele Frequency (Pro/Ala)	Odds ratio (OR)$^{\Psi}$; CI (95%) ProPro vs. ProAla +AlaAla	p-value
Probands[1]	353		289 (82)	64 (18)*	--	.909/.091	1.31; (0.92 – 1.86)	0.140
Affected[2] relatives	537		447 (83)	90 (17)*	--	.916/.084	1.48; (1.08 – 2.03)	0.016
Youngest[3] affected non-probands	205	44.22 ± 7.42	176 (86)	29 (14)	--	.929/.071	1.59; (0.95 – 2.59)	0.077
Oldest[4] affected non-probands	233	61.42 ± 9.89	187 (80)	46 (20)	--	.901/.099	1.22; (0.74 – 1.99)	0.431
Others affected[5] relatives	99	52.77 ± 10.97	85 (86)	14 (14)	--	.929/.071	1.45; (1.06 – 1.99)	0.020
Unaffected[6] relatives	296		249 (84)	47 (16)	--	.921/.079	--	--
Unrelated[7] controls	525		406 (77.3)	109 (20.8)*	10 (1.9)	.877/.122	1.00	--

[1]Index case representing each family; [2] affected sibling, parent or offspring, [3] youngest affected member representing each family (not including proband); [4] oldest affected member(sibling or parent, not including proband); [5] affected sibling, parent or offspring; [6] non-diabetic sibling, parent or offspring; [7] unrelated non-diabetic controls or spouse controls; Figures in parentheses are percent values; * significant difference ($p < 0.001$) between patients and controls; $^{\Psi}$ORs use the unrelated controls as reference group; $^{€}$ The ORs are adjusted for age, BMI, sex and waist circumference (WC).

Table 2: Distribution of PPARγ (P12A) polymorphism among SDS subjects and its association with T2DM.

	Probands		Affected Relatives					
			Youngest non proband Oldest non-proband				Others	
BMI (Age+Sex+ WC adjusted)	**Odds Ratio**	***p*-value**	**Odds Ratio**	***p*-value**	**Odds Ratio**	***p*-value**	**Odds Ratio**	***p*-value**
< 24.9 (Low risk)	1.39	0.282	1.27	0.630	1.66	0.195	1.13	0.649
>24.5 & < 27.5 (Increased risk)	1.30	0.151	1.49	0.119	1.16	0.555	1.41	0.034
>27.5 (High risk)	1.12	0.678	1.78	0.122	0.80	0.609	1.66	0.041
Waist Circumference (WC) (inches) (Age+Sex+BMI adjusted)								
< 34 cm (Low risk)	1.15	0.704	0.87	0.777	1.09	0.848	0.74	0.303
>34 & < 37.5 (Increased risk)	1.31	0.140	1.55	0.086	1.22	0.430	1.44	0.022
> 37 (High risk)	1.50	0.160	3.51	0.005	0.73	0.502	2.26	0.003
WHR (Age+Sex+BMI adjusted)								
<0.85 (Low risk)	2.03	0.527	0.24	0.136	1.21	0.849	0.59	0.404
>0.85 and <0.955 (Increased risk)	1.15	0.876	0.29	0.182	1.41	0.730	0.67	0.517
>0.95 (High risk)	1.67	0.039	3.02	0.003	1.21	0.605	2.15	0.001

¥Impact of PPARγ on T2DM was tested at each level of obesity adjusting for age, sex and other obesity traits using unrelated controls as reference group.

Table 3: Impact of PPARγ (P12A) polymorphism on T2DM susceptibility in relation to obesity¥.

	Combined Cases (890)		Proband (353)		Affected Relatives (537)		Unaffected Relatives (296)		Unrelated Controls (525)	
	Pro/Ala	**Pro/Ala**	**Pro/Pro**	**Pro/Ala**	**Pro/Pro**	**Pro/Ala**	**Pro/Pro**	**Pro/Ala**	**Pro/Pro**	**Pro/Ala**
BMI	27.57 ± 4.85 *P*=0.594	27.35 ± 4.14	27.37 ± 4.99 *P*=0.547	27.56 ± 3.95	27.70 ± 4.76 *P*=0.351	27.20 ± 4.28	27.38 ± 4.65 *P*=0.790	27.17 ± 5.09	26.10 ± 4.87 *P*=0.932	26.14 ± 5.10
Waist Circumference (WC)(cm)	37.30 ± 4.27 ***P*=0.028***	36.46 ± 4.31	37.19 ± 4.59 *P*=0.648	36.90 ± 4.02	37.36 ± 4.05 ***P*=0.011***	36.13 ± 4.50	36.12 ± 4.43 *P*=0.804	35.93 ± 4.84	35.21 ± 4.58 *P*=0.605	35.46 ± 4.82
WHR	0.973 ± 0.07 *P*=0.061	0.961 ± 0.08	0.970 ± 0.071 *P*= 0.884	0.967 ± 0.08	0.976 ± 0.071 ***P*=0.020***	0.956 ± 0.030	0.946 ± 0.075 *P*=0.574	0.937 ± 0.074	0.93 ± 0.075 *P*=0.204	0.94 ± 0.077

* Statistically significant difference between PPARγ genotypes.

Table 4: Obesity measures according to PPARγ genotypes among SDS Subjects.

(20.8% vs. 16%; P <0.001). The age, sex, WC and BMI- adjusted odds ratio (OR) associated with the more common 'Pro/Pro' genotype was 1.48 (95% CI; 1.08-2.03; P = 0.016) in affected relatives. This association remained marginally significant after we further stratified the affected relatives into youngest and oldest age categories. The ORs representing 'Pro/Pro' genotype-associated T2DM risk was 1.59 (95%CI; 0.95-2.59; P = 0.077) in youngest affected relatives and 1.45 (95%CI; 1.06-1.99; *P*=0.02) in other affected relatives; however, adjusted ORs were not significant in oldest affected relative group (1.22 (95% Cl; 0.74-1.99; P = 0.431). Clinical and biochemical attributes of patients and non-diabetic controls were also compared by genotype. No significant differences were observed in lipids, insulin, and creatinine levels between PPARγ genotypes (data not shown).

Relationship of PPARγ (P12A) polymorphism with obesity and insulin resistance

To analyze whether the T2DM risk associated with the P12A polymorphism was modulated by obesity, we assessed the genotypic distribution among cases and controls for quantitative traits for obesity (e.g. BMI, WC and WHR). As shown in the online Table 4, the mean

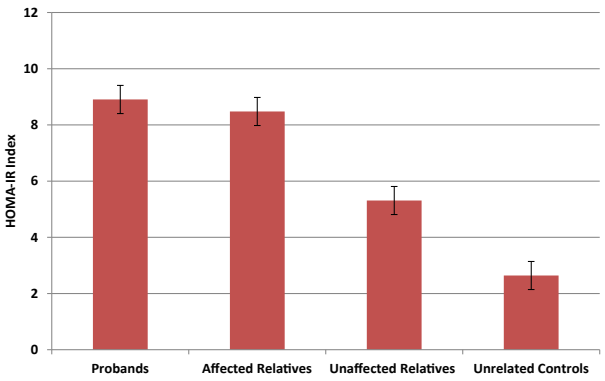

Figure 1: Mean values of HOMA IR among the members of SDS families stratified by disease. The difference between affected and unaffected groups was statistically significant (p<0.0001).

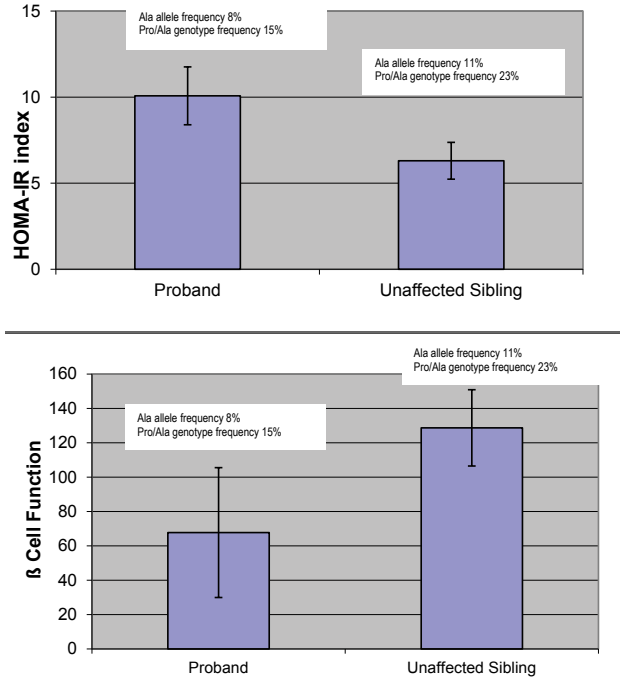

Figure 2: Mean values of HOMA IR and ß cell function among 246 discordant siblings stratified by PPARγ (P12A) genotypes.

BMI levels did not vary among genotypes across all groups. However, Pro/Ala genotype was associated with lower WC in combined cases (P=0.028) and in affected relatives (P=0.011) and with lower WHR (P=0.02) in affected relatives only. To further confirm whether the observed PPARγ (P12A) genotype-associated disease risk is modulated by central obesity, we modeled T2DM cases (separated by proband, youngest affected non-proband, oldest affected non-proband and other affected relatives) versus non-T2DM controls (unrelated) in subgroups defined by obesity related traits (BMI, WC and WHR) each separately. The values of each trait ranged from lowest, medium and highest defining 'low risk', 'increased risk' and 'high risk' categories, respectively. Because of the high prevalence of central adiposity in this population, the lower BMI cut off (for low risk) for the analysis was increased from 23 to 24.9 as very few individuals were present in the <23 BMI category. The risk estimates were calculated using Forward-Wald logistic regression analysis. As shown in Table 3, there was no association of this polymorphism with T2DM under low risk, overweight (increased risk) and obese (high risk) categories of BMI among probands. However, when we modeled the traits related to abdominal obesity (e.g. WC and WHR), the ORs for the PPARγ -associated risk were significant across the patients groups with WC >37cm or with WHR >0.95 (Table 3).

When we further compared age-matched discordant siblings (for T2D), the index of HOMA IR was significantly lower with higher ß cell function in unaffected siblings compared to probands (Figure 2). Interestingly, this also correlated well with the presence of higher frequency of protective Pro/Ala genotype (23% vs. 15%; P=0.075) and higher frequency of 'Ala' allele (11% vs. 8%; P=0.304) in unaffected (discordant) siblings compared to probands, the difference however, was not statistically significant because of small sample size of discordant sibling pairs (Figure 2).

Impact of PPARγ (P12A) on Age-of-Onset

To examine whether the PPARγ polymorphism has any impact on the onset of diabetes, we used Kaplan-Meier survival statistics to determine the effect of age-of-onset in all the affected subjects according to 'Pro/Pro' and 'Pro/Ala' genotypes in combined and gender stratified data. As shown in Figure 3, the 'Pro/Ala' genotypes revealed a marginally significant association with delayed age-of-onset compared to the Pro/Pro genotypes in males (P=0.035) but not in females (P=0.342).

Discussion

The goal of this study was to examine the role of P12A in the PPARγ gene on influencing the T2DM risk in a family- based sample of Khatri Sikhs. The P12A variant, present at the N-terminus region of PPARγ has been shown to confer significant risk against the development of T2DM [39]. Multiple GWAS and large meta-analysis studies using thousands of cases and controls have revealed a low odds ratio of 1.25 associated with Pro12 allele but with high population attributable risk of 25% [19,30,31,40]. The frequency of the 'Ala12' allele of the PPARγ polymorphism ranged between 7- 11 % in this entire SDS family sample. We were unable to detect any association of this polymorphism with T2DM when we compared the frequency among affected and unaffected relatives within families. However, the 'Pro/Pro' genotype was significantly associated with the disease when we analyzed the distribution affected relatives (OR 1.48; P=0.016) comparing with unrelated controls.

The lack of association within the family-based sample could be explained by several factors, including the possibility for overrepresentation of 'Pro12' allele in the family sample of this endogamous group due to inbreeding and selection, and unconfirmed disease status among unaffected younger relatives (especially <40 yrs) that could switch to T2DM after a period of time. To examine if the age of unaffected relatives was a contributing factor of difference in allele frequencies, we stratified the 'unaffected relatives' (n=296) into different age groups (29% were <40 yrs, 39% were between age 40-59 yrs, and about 32% were >60 yrs), but found no age-dependent allele frequency differences. Despite the absence of association of the PPARγ (P12A) polymorphism with T2DM risk in the family sample, our data indicate significant interaction of this polymorphism with central obesity and insulin sensitivity with stronger impact at younger age (Table 3 and Figure 2). One of the reasons for the observed lower impact of PPARγ in older age group (>50 yrs.) could be the confounding effect

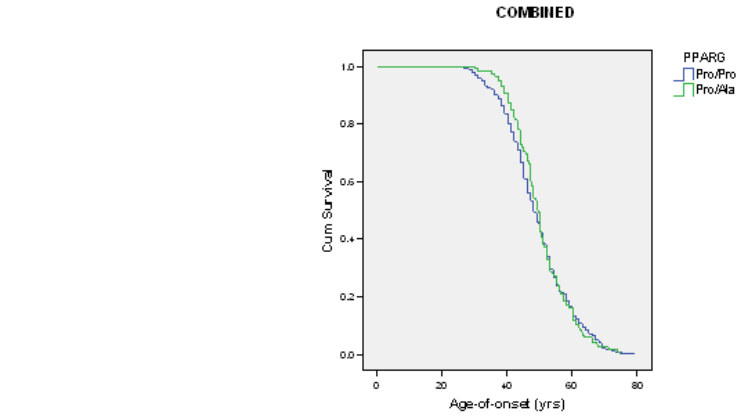

Figure3-A. All Patients; p=0.287

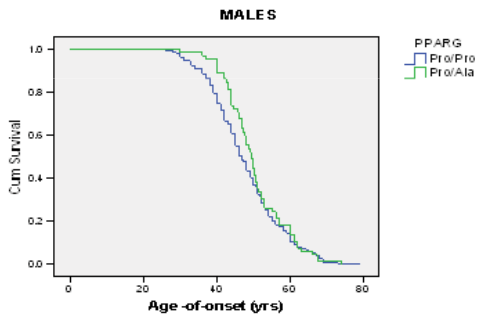

Figure 3-B. Males; p=0.035

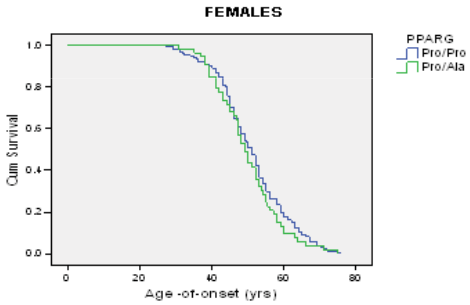

Figure3-C. Females; p=0.342

Figure 3: Kaplan-Meier survival curves showing the impact of PPARγ (P12A) genotype on age-of- onset in combined patients (males and females) (Figure 3-A) and data separated by gender (Figure 3-B and C). The p-values were derived using Wilcoxon statistics.

of lipid lowering drugs, ß- blockers, and other medications related to diabetic complications, which are used more commonly by older patients. Our findings agree with several other studies, where the obesity background has been shown to worsen the detrimental effect of 'Pro/Pro' genotype for increasing insulin sensitivity [41,42]. This also correlates well with high prevalence of insulin resistance and T2DM in this population with normal BMI. Following the new BMI thresholds based on the geographical regions [43], 82% of our family cohort is above the healthy BMl limits (23kg/m^2) and thus, is at increased risk of developing fat-related illness. As we reported before [23,43] in this population, obesity-related traits were present in both affected and unaffected members of the families. The clustering of these risk factors (e.g. triglycerides, WC, and HOMA IR) may influence higher insulin sensitivity among unaffected relatives. Possibly, the increased visceral fat in Asian Indians is not apparent from their non- obese BMI or overall degree of obesity. However, the computerized topographic (CT) multi-scan measures of body composition predict that Asian Indians have 30% more total body fat than age-matched African American men or White men [44,45] and 21% more total fat than Swedish men [46] in separate studies. Because of the presence of higher body fat with normal BMI, every small increase in the WC substantially increases their risk to insulin resistance and T2DM [3,47,48]. Moreover, the observed 'Pro/Ala' genotype-associated protection for delaying the T2DM onset in males (Figure 3) further suggests the role of this polymorphism in T2DM. Similar association of 'Ala/Ala' and 'Pro/Ala' genotypes with older age at onset has been recently reported in patients with multiple sclerosis [49]. However, more studies are needed to explain this gender-based association. Some earlier studies observed gender-specific association between 'Pro/Ala' genotype with HOMA IR and insulin sensitivity in either men [50] and women [31,42]. These inconsistencies indicate complex biological relationship of metabolic traits with PPARγ polymorphism [51].

A potential explanation for the observed differences could be partly governed by the differential expression of PPARγ in visceral and subcutaneous fat and different patterns of fat distribution among males and females. This could be mediated by the underlying intermediate metabolic pathways involving interactions with other gene(s) and environment(s) that may lead to the development of insulin resistance and T2DM. Earlier studies have also reported interaction of this polymorphism with obesity [52], diet [53], weight [54] or physical activity [55]. In view of the important role of PPARγ in the regulation of ß cell function and ß cell development [56], controlling insulin secretion in response to fatty acids [57] suggest in-depth investigations to discern the role of this locus in promoting insulin resistance through abdominal obesity.

In summary, we did not observe any association of the PPARγ polymorphism with T2DM in this family-based sample. However, a significant association was seen when the affected relatives were compared with unrelated controls. These findings are extension of our earlier studies where we reported a strong association of P12A with T2DM (OR 0.13; 95%CI 0.03-0.56; *P*= 0.0007) in an unrelated T2DM cohort from same Punjabi origin where this family sample was collected [23]. It is also noteworthy that with the exception of P12A, no other variant in this locus showed consistent association with T2DM among the 14 tag SNPs we studied previously [23]. Although, the

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