Single-nucleotide polymorphisms in peroxisome proliferator-activated receptor γ and their association with plasma levels of resistin and the metabolic syndrome in a South Indian population

ABDUL HASEEB[1], MOHAMMAD ILIYAS[1], SUBHABRATA CHAKRABARTI[2], ARIF A FAROOQUI[3], SUDHIR R NAIK[3], SUDIP GHOSH[1], MADHURI SURAGANI[1] and NASREEN Z EHTESHAM[1,*]

[1]*Molecular Biology Unit, National Institute of Nutrition, Jamai Osmania, Hyderabad 500 604, India*

[2]*L V Prasad Eye Institute, Banjara Hills, Hyderabad 500 034, India*

[3]*Mediciti Hospital, Hyderabad 500 063, India*

**Corresponding author (Fax, 91-40-27019074; Email, nasreen_e1@hotmail.com)*

Studies on the association of the Pro12Ala and C1431T polymorphisms of *PPARγ* with diabetes and obesity have revealed extensive population-dependent variations. However, association of these polymorphisms with the metabolic syndrome and its individual components has not been well investigated in the Indian population. The Indian population harbours the maximum number of diabetics in the world who are thus more susceptible to metabolic disorders. We screened a South Indian population ($N = 699$) for a possible association of these polymorphisms with the metabolic syndrome (MS) and type 2 diabetes. We also investigated the correlation of these two single-nucleotide polymorphisms (SNPs) with plasma resistin levels. The C1431T SNP was associated with higher levels of plasma resistin ($P = 0.017$). Furthermore, C1431T was associated with resistin in different tertiles. Prevalence of the 'Pro-C' haplotype decreased with increasing tertiles of resistin (84.1% to 75.4%, $P = 0.037$). Plasma resistin levels were not found to be associated with MS and type 2 diabetes. These results point to a likely association of plasma resistin levels with *PPARγ* polymorphisms in the Indian population.

[Haseeb A, Iliyas M, Chakrabarti S, Farooqui A A, Naik S R, Ghosh S, Suragani M and Ehtesham N Z 2009 Single-nucleotide polymorphisms in peroxisome proliferator-activated receptor γ and their association with plasma levels of resistin and the metabolic syndrome in a South Indian population; *J. Biosci.* **34** 405–414]

1. Introduction

The metabolic syndrome (MS), characterized by a clustering of common chronic degenerative disorders such as hypertension, type 2 diabetes mellitus (T2DM), dyslipidaemia and obesity (Alberti and Zimmet 1998), has a strong genetic component. Variations in several candidate genes have been widely implicated in predisposing to these disorders. Peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear hormone receptor, controls adipocyte differentiation (Kliewer *et al.* 1995) and regulates a number of genes associated with energy homeostasis (Tontonoz *et al.* 1995). Several studies on *PPARγ* polymorphisms in the context of different metabolic disorders have been conducted. The common variant Pro12Ala (rs1801282) of *PPARγ* has been widely studied across different

Keywords. C1431T; India; metabolic syndrome; Pro12Ala; resistin

Abbreviations used: BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; EM, expectation–maximization; HDL, high-density lipoprotein cholesterol; IDF, International Diabetes Federation; IGT, impaired glucose tolerance; IL, interleukin; IRS, insulin resistance syndrome; LD, linkage disequilibrium; MS, metabolic syndrome; NCEP ATP-III, National Cholesterol Education Program (NCEP) Adult Treatment Panel III; PCR, polymerase chain reaction; PPAR, peroxisome proliferator-activated receptor; PPREs, PPAR response elements; RFLP, restriction fragment length polymorphism; SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes mellitus; TNF, tumour necrosis factor

phenotypes, and varying degrees of association have been found (Stumvoll and Haring 2002). Though this variant has been consistently associated with T2DM among Caucasians (Altshuler *et al.* 2000; Stumvoll and Haring 2002), such associations have been inconsistent among Asians (Stumvoll and Haring 2002). In a Danish study on the MONICA cohort, homozygosity of the Ala allele was found to provide protection from the insulin resistance syndrome (IRS) (Frederiksen *et al.* 2002). In a large French cohort, a specific haplotype of the *PPARγ* gene was found to be associated with an increased risk for MS (Meirhaeghe *et al.* 2005). In a recent study on Chinese subjects, Pro12Ala polymorphism was associated with insulin resistance but showed no effect on the prevalence of MS as defined by the International Diabetes Federation (IDF) criteria (Dongxia *et al.* 2008). In another study on a large group of Swedish middle-aged subjects ($N = 5000$), the Pro12Ala SNP showed no association with MS defined according to the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III [ATP-III]) and IDF criteria (Montagnana *et al.* 2008). Another silent mutation in exon 6 of the *PPARγ* gene, C1431T (rs3856806), which has been studied in several populations, was found to be in linkage disequilibrium (LD) with the Pro12Ala SNP (Valve *et al.* 1999). In a French study, obese subjects exhibited an association with plasma leptin (Meirhaeghe *et al.* 1998), while in a European cohort, significantly higher high-density lipoprotein (HDL) cholesterol levels and lower body mass index (BMI) were found among individuals homozygous for the T allele (Doney *et al.* 2002). Two other studies on a large European cohort to study the impact of these single-nucleotide polymorphisms (SNPs) on obesity (Doney *et al.* 2002) and T2DM (Doney *et al.* 2004) have shown their modifying effect on each other, though the association of the haplotypes of these variants with MS has not yet been investigated. Rhee *et al.* (2006) have reported an association of Pro12Ala and C1431T SNPs with some parameters of MS but not with the overall prevalence of MS in Korean females ($N = 253$). Both the Pro12Ala and C1431T SNPs did not show any association with MS in a South Indian population from Chennai (Vimaleswaran *et al.* 2007).

PPAR*γ*, being a transcription factor, modulates the expression of several genes such as adiponectin, leptin and resistin, which are involved in fatty acid metabolism, glucose homeostasis and insulin sensitivity. Resistin is a small cysteine-rich secretory protein expressed in adipocytes and macrophages. It has been proposed that resistin provides a connecting link between obesity and insulin resistance (Steppan *et al.* 2001). In addition, it has also been shown to stimulate several pro-inflammatory cytokines including tumour necrosis factor (TNF)-*α* and interleukin (IL)-12 (Silswal *et al.* 2005). PPAR*γ* regulates the expression of resistin through several PPAR response elements (PPREs) present in the promoter region of the resistin gene (Patel *et al.* 2003). Since *PPARγ* polymorphisms have been shown to affect its transactivation (Deeb *et al.* 1998), they may also modulate the expression of genes regulated by this transcription factor. In a recent study on a small group of Chinese subjects ($N = 231$), plasma resistin levels were found to be lower in Ala carriers (Pro/Ala+Ala/Ala genotype) as compared with Pro homozygotes (Pro/Pro genotype) ($P<0.05$) (Wang *et al.* 2004).

Intriguingly, the prevalence of diabetes in Hyderabad (South India) is reported to be significantly high (16.6%) along with a higher impaired glucose tolerance (IGT) (29.8%) when compared with urban Indian populations living in other metropolitan cities (T2DM, 9.3–13.5%, IGT, 8.6–16.8%) (Ramachandran *et al.* 2001). Although Pro12Ala and the C1431T SNPs in *PPARγ* have been widely studied with respect to T2DM in the western and some Asian populations (Deeb *et al.* 1998; Doney *et al.* 2004; Tai *et al.* 2004), such studies are lacking in the Indian population, which harbours the highest number of people with diabetes (40.9 million of 246 million diabetics worldwide are from India alone; Sicree *et al.* 2006). A recent study on another South Indian population from Chennai failed to reveal any significant association of the Pro12Ala SNP with T2DM (Radha *et al.* 2006). In view of the contentious association of *PPARγ* SNPs with diabetes and MS, the present study was designed to evaluate the influence of the two variants of *PPARγ* on MS as an entity and its various components individually, in a South Indian population at high risk for these diseases. We also attempted to develop a correlation of these polymorphisms with serum resistin levels in metabolic disorders.

2. Materials and methods

2.1 *Subjects*

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Bioethics Committee of the National Institute of Nutrition. The cohort comprised 699 unrelated subjects who presented at the Mediciti Hospital, Hyderabad, India between August 2004 and August 2006. The demographic and clinical details of the subjects were documented through a pre-designed health questionnaire. MS as an entity defined by the NCEP ATP-III recommendations (Expert Panel 2001) includes at least three or more of the following abnormalities: waist circumference ≥90 cm in men or ≥80 cm in women (modified according to the recommendations of the IDF for South Asians), triglycerides ≥150 mg/dl, HDL cholesterol <40 mg/dl in men or <50 mg/dl in women, defined cases of hypertension, and defined cases of T2DM. According

to a recent definition by the IDF, subjects with a waist circumference ≥90 cm in men or ≥80 cm in women along with any two of the following abnormalities: triglycerides ≥150 mg/dl, HDL cholesterol <40 mg/dl in men or <50 mg/dl in women, defined cases of hypertension, and defined cases of T2DM; were classified as cases of MS (Zimmet *et al.* 2005). Subjects were diagnosed as having T2DM (mean age 61.8±11.25 years) based on their past history and being on antidiabetic or hypoglycaemic drugs. Controls enrolled were over 50 years of age (mean age 61.93±10.40 years) with normal glucose levels or elevated glucose levels without glycosylated haemoglobin. These subjects were further categorized based on hypertension and obesity. Hypertensive subjects were identified based on their use of antihypertensive drugs, while non-hypertensives were not on any antihypertensive medication and had normal systolic (127.61±24.36 mmHg) and diastolic (79.70±12.92 mmHg) blood pressure at three different time points recorded at an interval of 3 h. Subjects with a BMI cut-off value of 23 kg/m^2 and a waist circumference cut-off value of 80 cm for women and 90 cm for men were classified as obese, as described earlier for Asian Indian adults (Snehalatha *et al.* 2003; Zimmet *et al.* 2005).

Venous blood was collected from the subjects with prior informed written consent. Lipid parameters (HDL, triglycerides, total cholesterol) were estimated by the colorimetric method using reagents supplied by BioSystems (Spain). Plasma resistin level was measured with the human resistin enzyme-linked immunosorbent assay (ELISA) kit (AdipoGen, Inc., Seoul, Korea). To assess the effect of the genotypes of the Pro12Ala and C1431T SNPs and their haplotypes, the study subjects were classified into tertiles (of equal magnitude) based on plasma resistin levels using the following cut-off points: tertile 1, resistin levels ≤12 ng/ml; tertile 2, >12 ng/ml to ≤24 ng/ml; and tertile 3, >24 ng/ml.

2.2 *Genetic analysis*

Genomic DNA was extracted from whole blood using the MasterPure™ Genomic DNA purification kit (Epicentre, Madison, USA). The DNA fragments containing both the SNPs were amplified by polymerase chain reaction (PCR) with the following sets of primers (Pro12Ala: upstream 5′-GCC AAT TCA AGC CCA GTC C-3′, and a mutant downstream 5′-GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT CCT G-3′, which introduces a *Bsa*JI site; C1431T: upstream 5′-CCT CCC CAC CTA TTT AAG ATA-3′, downstream 5′-CGG GAT CCG TAC AAG TCC TTG TAG ATC TCC-3′ with a *Bsa*AI restriction enzyme site). The amplicons containing the SNPs were screened by restriction digestion with appropriate restriction enzymes, i.e. *Bsa*JI for Pro12Ala and *Bsa*AI for C1431T at appropriate temperatures for 2 h and resolved on a 2% agarose gel. Genotyping was repeated at least twice. The entire screening was done using PCR-restriction fragment length polymorphism (RFLP); only a subset of the genotypes was further confirmed by sequencing on an automated DNA sequencer (ABI 3100) using the BigDye chemistry following the manufacturer's guidelines (Applied Biosystems, Foster City, CA). The obtained sequences were analysed with the help of Sequencing software (version 3.0) and compared with the wild-type sequence obtained from GenBank. There was total concordance of genotype data obtained by restriction digestions and sequencing.

2.3 *Statistical analysis*

Allele and genotype frequencies were estimated by the allele counting method. Hardy–Weinberg estimates for genotypes and estimated haplotype frequencies were calculated using the HAPLOVIEW software that uses the expectation–maximization (EM) algorithm (Barrett *et al.* 2005). Linkage disequilibrium between the two SNPs was analysed using the LD plot function of this software. Both SNPs and their haplotypes were also compared across all the three tertiles of resistin levels. The Chi-square test was used to compare the genotype and allele frequencies between study groups. Odds ratios were computed for estimating the risk of T2DM, hypertension, BMI and waist circumference with respect to the different genotypes. The clinical covariates were adjusted for age and gender. Univariate analysis was carried out to check for the association of different genotypes with diabetes and hypertension using the SPSS software.

3. Results

The characteristics of the study subjects stratified according to their MS and non-MS status, as defined by NCEP ATP-III and IDF criteria, are displayed in table 1. All the anthropometric and lipid profile values were significantly different among these groups (table 1). However, no significant difference in plasma resistin levels was observed among MS and non-MS subjects with either of the two criteria. Univariate analysis of different genotypes did not show any effect on the clinical variables for diabetes and hypertension (table 2). However, the variant genotype (C/T+T/T) of C1431T was significantly associated with higher levels of plasma resistin (P=0.017) (table 2).

There was no significant deviation from the Hardy–Weinberg equilibrium among the subjects with respect to both the SNPs (P>0.05). The distributions of allele and genotype and haplotype frequencies along with their odds

Table 1. General characteristics of the study subjects categorized as having MS or non-MS as defined by the NCEP ATP-III and IDF criteria

	NCEP-III			IDF		
	MS	Non-MS	*P*	MS	Non-MS	*P*
Number (699)	460	239	-	381	318	-
Men/women	271/189	154/85	-	198/183	227/91	-
Age (years)	61.56±10.84	62.41±10.81	NS	61.17±11.22	62.67±10.31	NS
Diabetic/non-diabetic	313/139	37/210	-	251/126	99/223	-
Hypertensive/normotensive 296/155	55/192	-	239/138	112/210	-	
BMI	26.33±5.50	24.15±6.07	<0.0001*	27.23±5.21	23.60±5.84	<0.0001*
Waist circumference (cm)	94.29±8.58	87.83±9.36	<0.0001*	96.08±7.90	87.24±8.73	<0.0001*
Waist/hip ratio	0.949±0.120	0.899±0.139	<0.0001*	0.956±0.120	0.903±0.135	<0.0001*
Total cholesterol (mg/dl)	285.52±253.55	188.51±117.80	<0.0001*	285.87±257.84	210.94±157.52	<0.0001*
HDL (mg/dl)	35.13±12.61	45.87±25.07	<0.0001*	35.29±13.02	43.18±23.01	<0.0001*
Total triglyceride (mg/dl)	216.69±103.08	137.46±55.95	<0.0001*	212.89±103.94	160.42±79.45	<0.0001*
Resistin (ng/ml)	12.19±7.30 (*N*=381)	11.10±6.00 (*N*=218)	NS	12.14±7.39 (*N*=323)	11.39±6.21 (*N*=276)	NS

NS, non-significant (*P*>0.05); NCEP ATP-III, National Cholesterol Education Program Adult Treatment Panel-III; IDF, International Diabetes Federation; MS, metabolic syndrome cases; non-MS, controls; BMI, body mass index; HDL, high-density lipoprotein cholesterol; *adjusted *P* value <0.001 (adjusted for age and gender).

Table 2. General characteristics of the subjects according to the genotypes of Pro12Ala and C1431T polymorphisms

	Pro12Ala			C1431T		
	Pro/Pro	Pro/Ala, Ala/Ala	*P*	C/C	C/T, T/T	*P*
Number (699)	538 (77.0%)	149 (21.3%), 12 (1.7%)	-	513 (73.4%)	173 (24.8%), 13 (1.9%)	-
Men/women	325/213	93/56, 7/5	NS	317/196	100/73, 8/5	NS
Age (years)	62.07±10.25	61.19±12.58	NS	61.81±10.32	62.00±12.15	NS
BMI	25.71±5.74	25.06±5.97	NS	25.60±5.76	25.43±5.90	NS
Waist circumference (cm)	92.26±9.48	91.19±8.05	NS	91.94±9.52	92.20±9.00	NS
Waist/hip ratio	0.933±0.128	0.927±0.135	NS	0.9319±0.1294	0.9306±0.1294	NS
Systolic BP (mmHg)	134.22±26.26 (*N* =301)	138.02±28.19 (*N* =97)	NS	133.64±26.36 (*N* =290)	139.17±27.53 (*N* =108)	NS
Diastolic BP (mmHg)	83.88±14.30 (*N* =301)	84.21±12.73 (*N* =97)	NS	83.60±14.50 (*N* =290)	84.94±12.22 (*N* =108)	NS
Total cholesterol (mg/dl)	242.32±201.66	283.35±274.5	NS	246.94±210.50	263.99±247.61	NS
HDL (mg/dl)	38.76±18.57	39.47±19.29	NS	37.83±18.32	40.99±19.74	NS
Total triglyceride (mg/dl)	186.77±97.62	195.04±94.84	NS	190.16±100.49	184.95±86.78	NS
Resistin (ng/ml) (*N*=599)	11.62±6.68 (*N* =463)	12.42±7.49 (*N* =136)	NS	11.37±6.6 (*N* =441)	12.99±7.46 (*N* =158)	0.0108

NS, non-significant (*P*>0.05); BMI, body mass index; HDL, high-density lipoprotein cholesterol.

ratios among MS and non-MS subjects are presented in table 3. No association was observed for the Pro12Ala and C1431T SNPs between MS and non-MS subjects based on either of the criteria.

Table 4 shows the allele, genotype and haplotype frequencies of Pro12Ala and C1431T SNPs among diseased and control groups based on T2DM, hypertension and obesity (as defined by BMI and waist circumference). Allele frequencies were not significantly different among the phenotype categories. Mutant and heterozygous genotypes for both the SNPs were pooled with respect to the wild type to avoid vagaries of small sample sizes in

Table 3. Allele, genotype and haplotype frequencies of Pro12Ala and C1431T variants with respect to MS and non-MS as defined by the NCEP ATP-III and IDF criteria

	NCEP ATP-III			IDF		
	MS (*N*=460)	NMS (*N*=239)	OR (95%CI)	MS (*N*=381)	NMS (*N*=318)	OR (95%CI)
Pro12Ala						
Pro/Pro	348 (75.7%)	190 (79.5%)	-	289 (75.9%)	249 (78.3%)	-
Pro/Ala+Ala/Ala	112 (24.3%)	49 (20.5%)	0.80	92 (24.1%)	69 (21.7%)	0.87
			(0.55–1.17)			(0.61–1.24)
Ala allele	13.3%	10.7%		13.0%	11.6%	
C1431T						
CC	330 (71.7%)	183 (76.6%)	-	273 (71.7%)	240 (75.5%)	-
CT+TT	130 (28.3%)	56 (23.4%)	0.78	108 (28.3%)	78 (24.5%)	0.82
			(0.54–1.11)			(0.59–1.15)
T allele	15.1%	12.5%		14.8%	13.5%	
Haplotypes			*P* value			*P* value
Pro-C	81.5%	85.1%	0.097	81.7%	84.0%	0.268
Ala-T	10.0%	8.2%	0.278	9.6%	9.1%	0.790
Pro-T	5.2%	4.3%	0.477	5.3%	4.4%	0.440
Ala-C	3.3%	2.4%	0.357	3.4%	2.5%	0.305

NCEP ATP-III, National Cholesterol Education Program Adult Treatment Panel-III; IDF, International Diabetes Federation; MS, metabolic syndrome cases; NMS, controls.

each category. While the disease odds for the genotypes were not significant among most of these phenotype categories, a mild association was observed for the carriers of the mutant 'T' allele of C1431T (OR, 1.41, *P*=0.047) with respect to hypertension (table 4). Linkage disequilibrium (LD) between these two SNPs was almost similar across different phenotype categories such as diabetes (D'=0.719), hypertension (D'=0.713), BMI (D'=0.713) and waist circumference (D'=0.716). Four different haplotypes were generated for these SNPs with respect to these phenotype categories. The estimated haplotype frequencies were not significantly different among the cohorts with and without the clinical traits within these phenotype categories (table 4).

To assess the effect of the two polymorphisms on plasma resistin levels, subjects were grouped into tertiles. As shown in table 5, there was a significantly higher representation of the C allele in first tertile (T1) compared with the third tertile (T3) alone (T1 vs T3, OR [95% CI] 1.99 [1.04–3.81], *P*=0.0349) as well as with the second (T2) and third tertile combined (T1 vs T2+T3, OR [95% CI] 1.48 [1.07–2.15], *P*=0.0349).

Comparison of the four haplotypes (Pro-C; Pro-T; Ala-C and Ala-T) among the different tertiles of resistin level indicated a trend of decreasing prevalence of the 'Pro-C' haplotype (comprising the wild-type alleles of the two SNPs) in ascending tertiles. The Pro-C haplotype was significantly higher in T1 compared with T3 (*P*=0.037). The differences for the 'Pro-C' haplotype between T1 and T2 or T2 and T3 were not significant. The 'Pro-T' and 'Ala-T' haplotypes showed an increase in frequency in ascending tertiles T3>T2>T1 although this was statistically not significant (table 5).

4. Discussion

The primary goal of this study was to evaluate the impact of *PPARγ* polymorphisms and their haplotypes on MS and its individual components such as obesity, T2DM, hypertension and dyslipidaemia. The implications of these polymorphisms and their haplotypes on the level of resistin were assessed in a cohort-based study.

On a par with global trends, fast demographic changes, changes in lifestyle due to urbanization and a possible genetic susceptibility, the urban Indian population is at higher risk for developing MS. Recent surveys have reported a varied prevalence of MS in the Indian population depending on the region and socioeconomic status of the subjects (Ramachandran *et al.* 2003; Misra and Vikram 2004). A countrywide survey carried out in the metropolitan cities documented a significantly higher (16.6%) prevalence of T2DM in Hyderabad (South India) with higher IGT levels (29.8%) as compared with urban Indian populations living in other metropolitan cities (Ramachandran *et al.* 2001). In contrast to western populations where the Ala allele of *PPARγ* was found to exhibit a population-attributable risk

Table 4. Allele, genotype and haplotype frequencies of Pro12Ala and C1431T variants with respect to type 2 diabetes, hypertension, body mass index (BMI) and waist circumference (WC)

	Diabetics (N=350)	Non-diabetics (N=349)	OR (95%CI)	Hyper-tensives (N=351)	Normo-tensives (N=348)	OR (95%CI)	BMI≥23 (*N*=453)	BMI<23 (*N* =246)	OR (95%CI)	High WC (*N* =500)	Low WC (*N* =199)	OR (95%CI)
Pro12Ala												
Pro/Pro	76.3%	77.7%	-	74.4%	79.6%	-	78.1%	74.8%	-	77.3%	76.4%	-
Pro/Ala+ Ala/Ala	23.7%	22.3%	1.08 (0.76–1.54)	25.6%	20.4%	1.34 (0.95–1.92)	21.9%	25.2%	0.83 (0.58–1.19)	22.7%	23.6%	0.96 (0.65–1.40)
Ala allele	12.9%	11.9%		13.7%	11.1%		12.1%	12.8%		12.3%	12.6%	
C1431T												
C/C	71.7%	75.0%	-	70.0%	76.7%	-	74.0%	72.4%	-	72.2%	76.4%	–
C/T+T/T	28.2%	25.0%	1.19 (0.85–1.66)	30.0%	23.3%	1.41 (1.0–1.97)	26.0%	27.6%	0.92 (0.65–1.31)	27.8%	23.6%	1.25 (0.85–1.80)
T allele	15.6%	12.3%		15.8%	12.3%		14.0%	14.6%		14.7%	13.1%	
Haplotypes			*P* value			*P* value			*P* value			*P* value
Pro-C	82.0%	83.4%	0.485	81.8%	83.9%	0.278	82.8%	82.7%	0.984	81.9%	83.9%	0.342
Ala-T	9.7%	8.9%	0.598	10.3%	8.0%	0.131	8.7%	10.2%	0.371	9.5%	8.6%	0.579
Pro-T	5.6%	4.5%	0.341	5.1%	4.9%	0.878	5.2%	4.4%	0.517	6.0%	3.9%	0.083
Ala-C	2.7%	3.2%	0.561	2.8%	3.2%	0.723	3.2%	2.6%	0.516	2.5%	3.6%	0.276

Table 5. Genotype and haplotype frequencies in tertiles according to plasma resistin levels

	Pro12Ala		C1431T	
	Pro/Pro	X/Ala	C/C	X/T
	(*N*=463)	(*N* =136)	(*N* =441)	(*N* =158)
T1	287 (62%)	81 (59.6%)	282 (64%)	86 (54.4%)
T2	144 (31%)	42 (31%)	131 (29.7%)	55 (34.8%)
T3	32 (7%)	13 (9.6%)	28 (6.3%)	17 (10.8%)
	P>0.05 for all the groups		*OR (95% CI)*	
			$T1_{C/C}$ vs $T2+T3_{C/C}$: *1.48 (1.07–2.15), P=0.0349*	
			$T1_{C/C}$ vs $T3_{C/C}$: *1.99 (1.04–3.81),P =0.0349*	
Haplotypes				
	Pro-C	Ala-T	Pro-T	Ala-C
T1	84.1%	8.6%	4.1%	3.3%
T2	82.4%	9.8%	5.5%	2.3%
T3	75.4%	12%	8%	4.6%
	P=0.037 for $T1_{Pro-C}$ vs $T3_{Pro-C}$			

reduction of 20% in T2DM (Ek *et al.* 1999; Altshuler *et al.* 2000; Stumvoll and Haring 2002), most of the studies on Asian populations, including a South Indian population from Chennai, did not reveal any significant association (Radha *et al.* 2006).

In this study, we investigated the association of the Pro12Ala and C1431T SNPs and their haplotypes with the levels of resistin and MS in an Indian population. Subject classification in this study was done keeping in mind the unique central obesity phenotype of Indians.

No association of these SNPs and their haplotypes was found with MS (based on both the NCEP ATP-III and IDF critera) (table 3). Our findings are similar to earlier reports where no significant association of the SNPs of *PPARγ* could be established in other ethnic groups comprising Swedish (Montagnana *et al.* 2008), French (Meirhaeghe *et al.* 2005), Chinese (Dongxia *et al.* 2008), Danish MONICA cohort (Frederiksen *et al.* 2002) and another south Indian population from Chennai (Vimaleswaran *et al.* 2007).

The frequency of the minor Ala allele in this cohort (12.8%) was comparable with that in Caucasians (12.0% (Altshuler *et al.* 2000). However, this frequency was significantly higher than that in other Asian populations from Taiwan (4.0%), Korea (4.1%) (Oh *et al.* 2000), Malaysia (3.2%) (Tai *et al.* 2004), China (1.0%) and Japan (3.0%) (Mori *et al.* 1998). In contrast, the frequency of the T allele in our cohort (14.2%) was similar to that in Caucasian populations from the UK (11.9%) and France (13.3%) (Meirhaeghe *et al.* 1998; Doney *et al.* 2004) but was much lower than that in other Asian populations from Malaysia (22.0%) and China (25.2%) (Tai *et al.* 2004), pointing to the genetic closeness of the Indian population to Caucasians, at least with respect to the Ala allele and T allele. Though we did not find any association of C1431T with T2DM, BMI and central obesity, there was a mild association with hypertension (OR 1.41, *P*=0.047) (table 4). Interestingly, while the allele frequency of both the variants in Indians is similar to that in Caucasians, the implications of this are very different. This may be due to other genetic variants in candidate genes that are yet uncharacterized or interaction of genetic factors with environmental variants contributed by a difference in lifestyle. A recent study showed an inverse relationship between Ala frequency and T2DM in a population where the total energy intake from lipids exceeded 30% (Scacchi *et al.* 2007). Energy provided by lipids in the Indian population is surprisingly one of the lowest globally (~18%).

The strong LD observed for the Pro12Ala and C1431T SNPs in the present study, which was also seen in Indians living in Singapore (Tai *et al.* 2004), could be a function of the higher frequency of the mutant alleles in these two SNPs among Indians. Unlike that observed in a large diabetic cohort from Scotland, where haplotypes with the Ala allele and C allele of C1431T conferred a greater protection as opposed to Ala with the T allele (Doney *et al.* 2004), our study did not show any association of the haplotypes of these alleles with any significant risk or protection with respect to T2DM (table 4). Despite a strong LD between these SNPs, no significant association of any haplotype with BMI was observed (table 4), which is similar to the findings in Caucasians and migrant Indians in Singapore (Doney *et al.* 2002, 2004; Tai *et al.* 2004).

PPAR*γ* is a transcription factor regulating the expression of several adipokines. It has been shown that Pro12Ala and Pro115Gln affect the transactivation of PPAR*γ* *in vitro* (Deeb *et al.* 1998; Ristow *et al.* 1998). A Pro12Ala

substitution in PPARγ2 was associated with decreased receptor activity, whereas Pro115Gln showed increased transcriptional activity through blocking an inhibitory phosphorylation at an adjacent Serine (at position 114) (Ristow *et al.* 1998). Several PPARγ-binding sites (PPREs) have been shown to be present in the regulatory region of the human and mouse resistin gene (Ghosh *et al.* 2003; Patel *et al.* 2003). These PPREs have been predicted to be involved in the differential functioning of the human and mouse resistin gene (Ghosh *et al.* 2003; Patel *et al.* 2003). Though resistin has been proposed to be a connecting link between obesity and insulin resistance in rodents (Steppan *et al.* 2001), the role of resistin with respect to T2DM and insulin resistance in humans has been a subject of intense debate. While several studies have investigated its role in T2DM (Nagaev and Smith 2001) and obesity (Way *et al.* 2001), there are not many reports in relation to MS. In this study, we investigated the effect of Pro12Ala and C1431T SNPs on plasma resistin levels in an Indian population, in the context of metabolic disorders. Although higher levels of resistin have been recently found to be positively associated with insulin resistance (Hivert *et al.* 2008), we did not observe any difference in resistin levels in subjects classified on the basis of T2DM, obesity and MS. Moreover, while resistin levels were similar among subjects grouped in the Pro12Ala genotypes, it was significantly higher among carriers of the T allele of the C1431T polymorphism (P=0.017) (table 2). A genotype frequency comparison of both polymorphisms in tertiles of resistin levels revealed a significant association with the C1431T SNP (P=0.0349) (table 5). The prevalence of the 'Pro-C' haplotype (comprising the wild-type allele of both SNPs) was significantly lower in the third tertile of resistin (table 5). Therefore, it would be interesting to further investigate the impact of C1431T on resistin levels in other populations.

The main feature of this study was to understand the involvement of the two widely studied *PPARγ* SNPs in MS and related disorders such as T2DM, hypertension and obesity in a large cohort from South India. Second, apart from analysing the genotypes, we also explored the association of *PPARγ* haplotypes with plasma resistin levels in this cohort; this needs to be replicated further. While there could be certain limitations in using a hospital-based sample and issues pertaining to population substructuring, every effort was made to match the subjects with respect to their geographical region of origin and ethnicity. While there could be some inherent biases due to the vagaries of sample sizes in each phenotype category, the present data nevertheless represent a preliminary observation of *PPARγ* SNPs drawn from a potentially random population.

In conclusion, the present data indicate that: (i) both the Pro12Ala and C1431T variants of *PPARγ* do not exhibit any association with MS, T2DM and obesity; (ii) the C1431T SNP, however, shows a marginal association with hypertension; (iii) the 'T' allele of C1431T is associated with an increased level of plasma resistin; (iv) the 'Pro-C' haplotype is significantly lower in the third tertile of resistin; and (v) plasma resistin levels do not show association with MS and its individual components.

Acknowledgements

AH was supported by a fellowship from the Council of Scientific and Industrial Research, New Delhi. This work was supported by a grant to NZE from the Indian Council of Medical Research, New Delhi and was also partly supported by an SERC Fast Track Grant from the Department of Science and Technology to SG. The authors thank Sher Ali, National Institute of Immunology, New Delhi, for a critical review of this manuscript.

References

Alberti K G and Zimmet P Z 1998 Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation; *Diabet. Med.* **15** 539–553

Altshuler D, Hirschhorn J N, Klannemark M, Lindgren C M, Vohl M C, Nemesh J, Lane C R, Schaffner S F *et al.* 2000 The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes; *Nat. Genet.* **26** 76–80

Barrett J C, Fry B, Maller J and Daly M J 2005 Haploview: analysis and visualization of LD and haplotype maps; *Bioinformatics* **21** 263–265

Deeb S S, Fajas L, Nemoto M, Pihlajamaki J, Mykkänen L, Kuusisto J, Laakso M, Fujimoto W *et al.* 1998 A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity; *Nat. Genet.* **20** 284–287

Doney A, Fischer B, Frew D, Cumming A, Flavell D M, World M, Montgomery H E, Boyle D *et al.* 2002 Haplotype analysis of the PPARgamma Pro12Ala and C1431T variants reveals opposing associations with body weight; *BMC Genet.* **3** 21–28

Doney A S, Fischer B, Cecil J E, Boylan K, McGuigan F E, Ralston S H, Morris A D and Palmer C N 2004 Association of the Pro12Ala and C1431T variants of PPARG and their haplotypes with susceptibility to type 2 diabetes; *Diabetologia* **47** 555–558

Dongxia L, Qi H, Lisong L and Jincheng G 2008 Association of peroxisome proliferator-activated receptor gamma gene Pro12Ala and C161T polymorphisms with metabolic syndrome; *Circ. J.* **72** 551–557

Ek J, Urhammer S A, Sorensen T I, Andersen T, Auwerx J and Pedersen O 1999 Homozygosity of the Pro12Ala variant of the peroxisome proliferation activated receptor-gamma2 (PPAR-gamma2): divergent modulating effects on body mass index in obese and lean Caucasian men; *Diabetologia* **42** 892–895

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001 Executive summary of the

third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III); *JAMA* **285** 2486–2497

Frederiksen L, Brodbaek K, Fenger M, Jorgensen T, Borch-Johnsen K, Madsbad S and Urhammer S A 2002 Comment: studies of the Pro12Ala polymorphism of the PPAR-gamma gene in the Danish MONICA cohort: homozygosity of the Ala allele confers a decreased risk of the insulin resistance syndrome; *J. Clin. Endocrinol. Metab.* **87** 3989–3992

Ghosh S, Singh A K, Aruna B, Mukhopadhyay S and Ehtesham N Z 2003 The genomic organization of mouse resistin reveals major differences from the human resistin: functional implications; *Gene* **305** 27–34

Hivert M F, Sullivan L M, Fox C S, Nathan D M, D'Agostino R B Sr, Wilson P W and Meigs J B 2008 Association of adiponectin, resistin and TNF{alpha} with insulin resistance; *J. Clin. Endocrinol. Metab.* **93** 3165–3172

Kliewer S A, Lenhard J M, Willson T M, Patel I, Morris D C and Lehmann J M 1995 A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation; *Cell* **83** 813–819

Meirhaeghe A, Cottel D, Amouyel P and Dallongeville J 2005 Association between peroxisome proliferator-activated receptor gamma haplotypes and the metabolic syndrome in French men and women; *Diabetes* **54** 3043–3048

Meirhaeghe A, Fajas L, Helbecque N, Cottel D, Lebel P, Dallongeville J, Deeb S, Auwerx J *et al.* 1998 A genetic polymorphism of the peroxisome proliferator-activated receptor gamma gene influences plasma leptin levels in obese humans; *Hum. Mol. Genet.* **7** 435–440

Misra A and Vikram N K 2004 Insulin resistance syndrome (metabolic syndrome) and obesity in Asian Indians: evidence and implications; *Nutrition* **20** 482–491

Montagnana M, Fava C, Nilsson P M, Engström G, Hedblad B, Lippi G, Minuz P, Berglund G *et al.* 2008 The Pro12Ala polymorphism of the PPARG gene is not associated with the metabolic syndrome in an urban population of middle-aged Swedish individuals; *Diabet. Med.* **25** 902–908

Mori Y, Kim-Motoyama H, Katakura T, Yasuda K, Kadowaki H, Beamer B A, Shuldiner A R, Akanuma Y *et al.* 1998 Effect of the Pro12Ala variant of the human peroxisome proliferator-activated receptor gamma 2 gene on adiposity, fat distribution, and insulin sensitivity in Japanese men; *Biochem. Biophys. Res. Commun.* **251** 195–198

Nagaev I and Smith U 2001 Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle; *Biochem. Biophys. Res. Commun.* **285** 561–564

Oh E Y, Min K M, Chung J H, Min Y K, Lee M S, Kim K W and Lee M K 2000 Significance of Pro12Ala mutation in peroxisome proliferator-activated receptor-gamma2 in Korean diabetic and obese subjects; *J. Clin. Endocrinol. Metab*. **85** 1801–1804

Patel L, Buckels A C, Kinghorn I J, Murdock P R, Holbrook J D, Plumpton C, Macphee C H and Smith S A 2003 Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators; *Biochem. Biophys. Res. Commun.* **300** 472–476

Radha V, Vimaleswaran K S, Babu H N, Abate N, Chandalia M, Satija P, Grundy S M, Ghosh S *et al.* 2006 Role of genetic polymorphism peroxisome proliferator-activated receptor-gamma2 Pro12Ala on ethnic susceptibility to diabetes in South-Asian and Caucasian subjects: evidence for heterogeneity; *Diabetes Care* **29** 1046–1051

Ramachandran A, Snehalatha C, Kapur A, Vijay V, Mohan V, Das A K, Rao P V and Yajnik C S and Diabetes Epidemiology Study Group in India (DESI) 2001 High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey; *Diabetologia* **44** 1094–1101

Ramachandran A, Snehalatha C, Satyavani K, Sivasankari S and Vijay V 2003 Metabolic syndrome in urban Asian Indian adults – a population study using modified ATP III criteria; *Diabetes Res. Clin. Pract.* **60** 199–204

Rhee E J, Oh K W, Lee W Y, Kim S Y, Oh E S, Baek K H, Kang M I and Kim S W 2006 Effects of two common polymorphisms of peroxisome proliferator-activated receptor-gamma gene on metabolic syndrome; *Arch. Med. Res.* **37** 86–94

Ristow M, Müller-Wieland D, Pfeiffer A, Krone W and Kahn C R 1998 Obesity associated with a mutation in a genetic regulator of adipocyte differentiation; *N. Engl. J. Med.* **339** 953–959

Scacchi R, Pinto A, Rickards O, Pacella A, De Stefano G F, Cannella C and Corbo R M 2007 An analysis of peroxisome proliferator-activated receptor gamma (PPAR-gamma 2) Pro12Ala polymorphism distribution and prevalence of type 2 diabetes mellitus (T2DM) in world populations in relation to dietary habits; *Nutr. Metab. Cardiovasc. Dis.* **17** 632–641

Sicree R, Shaw J and Zimmet P 2006 Diabetes and impaired glucose tolerance; in *Diabetes atlas* 3rd edition (ed.) D Gan (Belgium: International Diabetes Federation) pp 15–103

Silswal N, Singh A K, Aruna B, Mukhopadhyay S, Ghosh S and Ehtesham N Z 2005 Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway; *Biochem. Biophys. Res. Commun.* **334** 1092–1101

Snehalatha C, Viswanathan V and Ramachandran A 2003 Cutoff values for normal anthropometric variables in Asian Indian adults; *Diabetes Care* **26** 1380–1384

Steppan C M, Bailey S T, Bhat S, Brown E J, Banerjee R R, Wright C M, Patel H R, Ahima R S *et al.* 2001 The hormone resistin links obesity to diabetes; *Nature (London)* **409** 307–312

Stumvoll M and Haring H 2002 The peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism; *Diabetes* **51** 2341–2347

Tai E S, Corella D, Deurenberg-Yap M, Adiconis X, Chew S K, Tan C E and Ordovas J M 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

Tontonoz P, Hu E, Devine J, Beale E G and Spiegelman B M 1995 PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene; *Mol. Cell. Biol.* **15** 351–357

Valve R, Sivenius K, Miettinen R, Pihlajamaki J, Rissanen A, Deeb S S, Auwerx J, Uusitupa M *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

Vimaleswaran K S, Radha V, Deepa R and Mohan V 2007 Absence of association of metabolic syndrome with PPARGC1A, PPARG and UCP1 gene polymorphisms in Asian Indians; *Metab. Syndr. Relat. Disord.* **5** 153–162

Wang C, Zhai F, Chi Y and Wang G 2004 Association of Pro12Ala mutation in peroxisome proliferator-activated receptor gamma 2 with obesity and diabetes in Chinese population; *Wei Sheng Yan Jiu.* **33** 317–320

Way J M, Görgün C Z, Tong Q, Uysal K T, Brown K K, Harrington W W, Oliver W R Jr, Willson T M *et al.* 2001 Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists; *J. Biol. Chem*. **276** 25651–25653

Zimmet P, Magliano D, Matsuzawa Y, Alberti G and Shaw J 2005 The metabolic syndrome: a global public health problem and a new definition; *J. Atheroscler. Thromb*. **12** 295–300

MS received 3 December 2008; accepted 5 May 2009

*e*Publication: 17 July 2009

Corresponding editor: Partha P Majumder