Effect of polymorphisms in the *PPARGC1A* gene on body fat in Asian Indians

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Objective: To evaluate whether polymorphisms in the peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (*PPARGC1A*) gene were related to body fat in Asian Indians.

Methods: Three polymorphisms of *PPARGC1A* gene, the Thr394Thr, Gly482Ser and +A2962G, were genotyped on 82 type 2 diabetic and 82 normal glucose tolerant (NGT) subjects randomly chosen from the Chennai Urban Rural Epidemiology Study using PCR-RFLP, and the nature of the variants were confirmed using direct sequencing. Linkage disequilibrium (LD) was estimated from the estimates of haplotypic frequencies using an expectation–maximization algorithm. Visceral, subcutaneous and total abdominal fat were measured using computed tomography, whereas dual X-ray absorptiometry was used to measure central abdominal and total body fat.

Results: None of the three polymorphisms studied were in LD. The genotype (0.59 vs 0.32, $P=0.001$) and allele (0.30 vs 0.17, $P=0.007$) frequencies of Thr394Thr polymorphism were significantly higher in type 2 diabetic subjects compared to those in NGT subjects. The odds ratio for diabetes (adjusted for age, sex and body mass index) for the susceptible genotype, XA (GA + AA) of Thr394Thr polymorphism, was 2.53 (95% confidence intervals: 1.30–5.04, $P=0.009$). Visceral and subcutaneous fat were significantly higher in NGT subjects with XA genotype of the Thr394Thr polymorphism compared to those with GG genotype (visceral fat: XA 148.2±46.9 vs GG 106.5±51.9 cm^2, $P=0.001$; subcutaneous fat: XA 271.8±167.1 vs GG 181.5±78.5 cm^2, $P=0.001$). Abdominal (XA 4521.9±1749.6 vs GG 3445.2±1443.4 g, $P=0.004$), central abdominal (XA 1689.0±524.0 vs GG 1228.5±438.7 g, $P<0.0001$) and non-abdominal fat (XA 18763.8±8789.4 vs GG 13160.4±4255.3 g, $P<0.0001$) were also significantly higher in the NGT subjects with XA genotype compared to those with GG genotype. The Gly482Ser and +A2962G polymorphisms were not associated with any of the body fat measures.

Conclusion: Among Asian Indians, the Thr394Thr (G→A) polymorphism is associated with increased total, visceral and subcutaneous body fat.

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Introduction

Obesity has today become an alarming public health problem not only in the developed but also in developing countries.[1] Central body obesity is particularly important because of its association with diabetes, dyslipidemia, hypertension and cardiovascular disease.[2] Recent studies have shown that Asian Indians have a greater degree of insulin resistance,[3–6] and increased susceptibility to diabetes[7–10] compared to Europeans. The 'Asian Indian phenotype' refers to the fact that they have increased visceral fat and central body obesity despite low rates of obesity as defined by body mass index (BMI).[8–11] Computed tomography (CT) is a powerful imaging technique that has the ability to differentiate the mass of adipose tissue located at the abdominal level into visceral and subcutaneous fat. Various cutoff values have been proposed in different populations to predict diabetes and other morbidities.[11] Dual X-ray absorptiometry (DXA) is usually used to measure total body fat, but has also been used to measure central abdominal fat.[12] We have recently shown that visceral fat measured by CT and

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central abdominal fat measured by DXA are strongly associated with type 2 diabetes in urban Asian Indians.[13] While visceral fat is related to lifestyle factors like diet and exercise, there is also evidence to suggest that it could be influenced by genetic factors.[14] The Quebec and the HERITAGE family studies have provided heritability estimates for total abdominal fat, subcutaneous fat and visceral fat.[15,16] Segregation analyses of these two study samples have also provided evidence for the role of a single gene with a major effect on abdominal visceral fat.[15,16]

Peroxisome proliferator-activated receptor-γ coactivator-1 alpha (PPARGC1A) is a transcriptional coactivator that has been implicated in the regulation of genes involved in energy metabolism.[17,18] Studies in rodents and cell culture models show that PPARGC1A stimulates mitochondrial biogenesis and activates genes of the oxidative phosphorylation pathway and thermogenesis.[19] The chromosomal region (4p15.1), in which PPARGC1A gene is located, has been associated with basal insulin levels in Pima Indians[20] and abdominal subcutaneous fat in the Quebec family study.[21] Esterbauer *et al*[22] have also reported an association of +A2962G of the PPARGC1A polymorphism with obesity in middle-aged European women. Owing to these reported associations and the role of PPARGC1A in energy metabolism in animal models, we examined three commonly studied polymorphisms of PPARGC1A gene, namely the Thr394Thr (+1302G>A) silent polymorphism, the Gly482Ser (+1564G>A) polymorphism and the +A2962G polymorphism, and showed that the Thr394Thr, but not the other two polymorphisms, was associated with type 2 diabetes in Asian Indians.[23] In this paper, we report that the Thr394Thr silent polymorphism is also associated with increased body fat and this is the first report to our knowledge, demonstrating an association of Thr394Thr polymorphism in the PPARGC1A gene polymorphism with body fat in Asian Indians.

Methods

Subjects

This is a case–control study of diabetic and non-diabetic subjects selected from the Chennai Urban Rural Epidemiology Study (CURES), an ongoing epidemiology study conducted on a representative population (aged $\geqslant$20 years) of Chennai (formerly Madras) in Southern India. The methodology of CURES is published elsewhere.[24] Briefly, in Phase I of CURES, 26001 individuals were recruited based on a systematic random sampling technique. Self-reported diabetic subjects on drug treatment of diabetes were classified as 'known diabetic subjects'.

In Phase 2 of CURES, the known diabetic subjects ($n=1529$) were invited to visit the center for detailed studies. In addition, every 10th individual of the 26001 individuals screened in Phase 1 ($n=2600$) were invited to undergo oral glucose tolerance tests (OGTT) using 75 g oral glucose load (dissolved in 250 ml of water). Those who were confirmed by OGTT to have 2 h plasma glucose value $\geqslant$11.1 mmol/l (200 mg/dl) based on WHO consulting group criteria were labeled as 'newly detected diabetic subjects' and those with 2 h plasma glucose value <7.8 mmol/l (140 mg/dl) as having normal glucose tolerance (NGT).

Using computer-generated random numbers, 82 diabetic (males: 38; females: 44) and 82 age and sex-matched NGT (males: 38; females: 44) subjects were selected for this study. The diabetic group had 52 known and 30 newly diagnosed diabetic subjects. Genotyping was carried out for the three polymorphisms of PPARGC1A gene namely the Thr394Thr silent polymorphism, Gly482Ser polymorphism and +A2962G polymorphism, and these were correlated with clinical and metabolic parameters, particularly with abdominal fat distribution and total body fat as measured by CT and DXA scans. Informed consent was obtained from all study participants, and the study was approved by the institutional ethics committee.

The clinical and biochemical profiles of the study groups and the associations of visceral fat (measured by CT scan and central abdominal fat measured by DXA) with diabetes were reported by us earlier.[13] The present study deals with the association of body fat with the three polymorphisms of the PPARGC1A gene.

Body composition

Anthropometrics. Height was measured with a tape to the nearest centimeter. Subjects were requested to stand upright without shoes with their back against their wall, heels together and eyes directed forward. Weight was measured with traditional spring balance that was kept on a firm horizontal surface. The scale was checked everyday and calibration was carried out with 'known' weights. Subjects were asked to wear light clothing and weight was recorded to the nearest 0.5 kg. The BMI was calculated using the formula, weight (kg)/height (m^2). Waist was measured using a non-stretchable fiber measure tape. The participants were asked to stand erect in a relaxed position with both feet together and one layer of clothing was accepted.[25]

CT and DXA. Subcutaneous and visceral fat were measured using a Helical CT scan (General Electric, Milwaukee, WI, USA). The scans were carried out at 120 kV, 200–250 mA. The parameters studied included visceral, subcutaneous and total abdominal fat. Visceral fat was distinguished from subcutaneous abdominal fat by tracing along the fascial plane defining the internal abdominal wall. DXA was used to determine total body fat, abdominal fat, central abdominal fat and non-abdominal fat. Central abdominal fat was calculated by the construction of an abdominal window as described by Carey *et al*.[26] The upper margin of this window was fixed at the lower border of the second lumbar vertebra (L2) and the lower margin at the lower border of the fourth

lumbar vertebra (L4). The lateral margins were fixed in alignment with the outer edges of the ribcage so as to exclude most of the lateral subcutaneous fat. The machine used was a Lunar Prodigy (Model 8743-BX/1L; GE Lunar, Madison, WI, USA). Both these procedures were carried out on the same day by two different observers at Bharat Scans (Chennai, India) a specialized center for imaging and radiological studies. Both the observers and the radiologist who interpreted the scans were unaware of the clinical status of the study subjects. The details of the methodology of CT scan and DXA procedure are published elsewhere.[13]

Molecular analysis

EDTA-anticoagulated venous blood samples were collected from all study subjects and the genomic DNA was isolated from whole blood by proteinase K digestion followed by phenol–chloroform extraction.[27] Subsequently, genomic DNA was precipitated in ethanol. Detection of the three polymorphisms was carried out using an amplification and restriction enzyme digestion technique. The Thr394Thr polymorphism was genotyped employing restriction site-generating (RG) PCR (T_{anneal}53 °C) with upstream RG primer 5′-GCCAGTCAATTAATTCCAAACC-3′ and downstream primer 5′-TTGGAGCTGTTTTCTTGTGC-3′.[28] The Gly482Ser polymorphism (T_{anneal}55 °C) was amplified with the following set of primers: 5′-CAAGTCCTCAGTCCTCAC-3′ and 5′-GGGGTCTTTGAGAAAATAAGG-3′.[29] The +A2962G polymorphism (T_{anneal}60 °C) was genotyped using the primers: 5′- CAATAACAACAATGGTTTACATGA -3′ and 5′- GAA CATTTTGAAGTTCTAGGTTTTACG-3′. PCR products were digested overnight with 3 U of *Msp*I (New England Biolabs, USA) for Thr394Thr polymorphism, 2 U of *Hpa*II (Bangalore Genei, Bangalore, India) for Gly482Ser polymorphism and 2 U of *Mlu*I (New England Biolabs, USA) for +A2962G polymorphism, and the products were resolved by 3% agarose gel electrophoresis. The assays were performed by a technician who was blinded to the phenotype. To assure that the genotyping was of adequate quality, we performed random duplicates in 20% of the samples. Further the heterozygous variants of the three SNPs were confirmed using direct sequencing by ABI 310 genetic analyzer.

Statistical analysis

Statistical analyses were performed using SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, USA) software program version 10.0 for windows. Data are presented as mean ± s.d. The effects of the three polymorphisms on quantitative and categorical variables were analyzed. The homozygous variants were very few in number and hence were pooled with the heterozygous variants for each of the three polymorphisms for the following analyses. χ^2 goodness-of-fit test was used to test whether the cases and controls, separately, were in Hardy–Weinberg proportions at the screened polymorphisms. Student's *t*-test as appropriate was used to compare genotype groups for continuous variables after adjustment for age and gender. χ^2 test or Fisher's exact test, as appropriate, was used to compare the proportions. Linear regression models of obesity phenotypes controlling for diabetes status and logistic regression models of diabetes status controlling for measures of obesity were carried out. Linkage disequilibrium (LD) among the alleles of the three *PPARGC1A* gene polymorphisms was tested with an EM (Expectation- Maximization) algorithm.[30] Power was calculated using the website: http://www.dssresearch.com/toolkit/spcalc/power_a2.asp.

Results

Subject characteristics

The mean ages of diabetic and NGT subjects were 45 ± 9 and 45 ± 9 years, respectively. Compared to NGT subjects, the diabetic subjects had significantly higher BMI (diabetes: 26.1 ± 4.2 vs NGT: 24.0 ± 4.7 kg/m^2, $P = 0.003$) and waist circumference (diabetes: 92.3 ± 9.4 vs NGT: 87.2 ± 11.4 cm, $P = 0.002$). The mean duration of diabetes in known diabetic subjects was 5.5 ± 4.6 years and none had ketonuria nor a history of ketoacidosis. Among the 52 known diabetic subjects, four (7.7%) were on diet alone, 41 (78.8%) on oral hypoglycemic drugs (21 on sulfonylureas, 12 on metformin, eight on a combination of sulfonylurea and metformin), three (5.8%) on insulin and four (7.7%) on a combination of insulin and metformin.

Genotype and allele frequencies

The genotype and allele frequencies of the three polymorphisms are presented in Table 1. Each of the three marker loci was in Hardy–Weinberg equilibrium among both diabetic and NGT subjects. The polymorphisms were not in pairwise LD. The LD values between the loci Thr394Thr-Gly482Ser, Gly482Ser-+A2962G and Thr394Thr-+A2962G were 0.2435 ($P = 0.22$), 0.4325 ($P = 0.55$) and 0.4643 ($P = 0.85$), respectively, in the cases and 0.3361 ($P = 0.36$), 0.3285 ($P = 0.49$) and 0.3862 ($P = 0.89$), respectively in the controls.

With respect to Thr394Thr silent polymorphism, 58.5% of the type 2 diabetic patients (48/82) had the variant XA (GA + AA) genotype compared with 32.1% of the NGTs (27/82) ($P = 0.001$). The frequency of the 'A' allele was also higher among type 2 diabetic subjects (0.30) compared to NGT subjects (0.17, $P = 0.007$). The odds ratio (adjusted for age, sex and BMI) for diabetes for the susceptible genotype (XA) was 2.53 (95% confidence intervals: 1.30–5.04, $P = 0.009$). There were no statistically significant differences in the genotype distribution between the type 2 diabetic and NGT subjects with respect to the Gly482Ser and A2962G polymorphisms.

Association of PPARGC1A gene polymorphisms with body fat measurements

Table 2 shows the body fat measurements using CT classifying the study subjects based on the genotypes of Thr394Thr, Gly482Ser and +A2962G 3′-UTR polymorphisms of *PPARGC1A* gene. Total abdominal, visceral and subcutaneous fat were significantly higher in type 2 diabetic subjects with XA genotype of the Thr394Thr polymorphism compared to those with GG genotype (total abdominal fat: XA 405.3±129.0 vs GG 334.8±81.1 cm^2, $P=0.005$; visceral fat: XA 148.1±46.2 vs GG 130.9±31.4 cm^2 $P=0.040$; subcutaneous fat: XA 257.0±108.8 vs GG 203.2±72.0 cm^2 $P=0.005$). Similarly in NGT subjects, total abdominal, visceral and subcutaneous fat measured by CT were significantly higher in subjects with XA genotype compared to those with GG genotype (total abdominal fat: XA 428.7±168.6 vs GG 289.9±97.6 cm^2 $P<0.0001$; visceral fat: XA 148.2±46.9 vs GG 106.5±51.9 cm^2 $P=0.005$; subcutaneous fat: XA 271.8±167.1 vs GG 181.5±78.5 cm^2 $P<0.0001$). The *P*-values shown above for the obesity phenotypes are adjusted for age, sex and type 2 diabetes. There was no association between the Gly482Ser or +A2962G polymorphisms with either visceral or subcutaneous fat measured by CT.

Table 3 shows the associations between the three polymorphisms studied and total body fat and abdominal fat measured by DXA. Total body fat (XA 22233.0±7804.4 (33.3±7.6%) vs GG 18955.2±5689.5 (28.9±6.8%) g, $P=0.012$), central abdominal fat (XA 1622.2±460.2 vs GG 1477.6±311.4 g, $P=0.031$) and non-abdominal fat (XA 17603.0±6905.3 vs GG 14439.2±5083.8 g, $P=0.005$) were significantly higher in the type 2 diabetic subjects with XA genotype compared to those with GG genotype. Similarly, in NGT subjects, total body fat mass and % body weight (XA

Table 1 Genotypic and allelic frequencies at the three different polymorphisms of the *PPARGC1A* gene

Genotypes	Frequencies		Alleles	Frequencies	
	Type 2 diabetic subjects (n = 82)	NGT subjects (n = 82)		Type 2 diabetic subjects	NGT subjects
Thr394Thr (G→A) polymorphism					
GG	0.42	0.67	G	0.70	0.83
GA	0.57	0.32			
			A	0.30	0.17
AA	0.01	0.01			
Gly482Ser (G→A) polymorphism					
GG	0.46	0.58	G	0.72	0.78
GA	0.52	0.39			
			A	0.28	0.22
AA	0.02	0.03			
+A2962G (A→G) polymorphism					
AA	0.43	0.52	A	0.71	0.76
AG	0.56	0.47			
			G	0.29	0.24
GG	0.01	0.01			

The three genotype distributions are in Hardy–Weinberg equilibrium in cases and controls.

Table 2 Body fat measurements using CT scan for the study subjects classified based on the genotypes of Thr394Thr, Gly482Ser and +A2962G polymorphisms of *PPARGC1A* gene

	Type 2 diabetic subjects (n = 82)			NGT subjects (n = 82)		
	GG (n = 34)	XA (GA+AA) (n = 48)	P-value* (power)	GG (n = 55)	XA (GA+AA) (n = 27)	P-value* (power)
Thr394Thr polymorphism						
Total abdominal fat (cm^2)	334.8±81.1	405.3±129.0	0.005 (0.85)	289.9±97.6	428.7±168.6	<0.0001 (0.98)
Subcutaneous fat (cm^2)	203.2±72.0	257.0±108.8	0.005 (0.77)	181.5±78.5	271.8±167.1	<0.0001 (0.76)
Visceral fat (cm^2)	130.9±31.4	148.1±46.2	0.040 (0.56)	106.5±51.9	148.2±46.9	0.005 (0.96)
	Type 2 diabetic subjects (n = 82)			NGT subjects (n = 82)		
	Gly/Gly (n = 38)	X/Ser (Gly/Ser+Ser/Ser) (n = 44)	P-value* (power)	Gly/Gly (n = 48)	X/Ser (Gly/Ser+Ser/Ser) (n = 34)	P-value* (power)
Gly482Ser polymorphism						
Total abdominal fat (cm^2)	360.0±92.6	387.6±121.5	0.295 (0.21)	346.5±156.7	328.9±115.7	0.614 (0.09)
Subcutaneous fat(cm^2)	223.4±75.4	235.4±102.9	0.583 (0.09)	220.1±142.8	210.9±83.8	0.762 (0.07)
Visceral fat (cm^2)	136.0±34.2	151.2±42.1	0.109 (0.43)	120.9±54.0	117.2±52.9	0.774 (0.06)
	Type 2 diabetic subjects (n = 82)			NGT subjects (n = 82)		
	AA (n = 35)	XG (AG+GG) (n = 47)	P-value* (power)	AA (n = 43)	XG (AG+GG) (n = 39)	P-value* (power)
+A2962G 3′- UTR polymorphism						
Total abdominal fat (cm^2)	374.2±111.5	379.8±109.9	0.843 (0.06)	328.2±128.0	338.5±156.1	0.768 (0.06)
Subcutaneous fat (cm^2)	227.7±97.9	238.2±95.2	0.666 (0.08)	197.8±105.7	223.2±136.7	0.398 (0.16)
Visceral fat (cm^2)	145.5±40.9	141.1±33.0	0.643 (0.08)	124.7±57.9	114.3±51.0	0.426 (0.13)

Data are presented as mean±s.d. *P-value adjusted for age, sex and type 2 diabetes.

Table 3 Body fat measurements using DXA for the study subjects classified based on the genotypes of Thr394Thr, Gly482Ser and +A2962G polymorphisms of *PPARGC1A* gene

	Type 2 diabetic subjects (n = 82)			*NGT subjects (n = 82)*		
	GG (n = 34)	*XA (GA+AA) (n = 48)*	*P-value* (power)*	*GG (n = 55)*	*XA (GA+AA) (n = 27)*	*P-value* (power)*
Thr394Thr polymorphism						
Total body fat (g) (body fat %)	18955.2±5689.5 (28.9±6.8)	22233±7804.4 (33.3±7.6)	0.012 (0.59)	16601.0±5177.1 (29.1±6.1)	23 285.7±10 229.5 (34.1±8.7)	<0.0001 (0.89)
Abdominal fat (g)	4245.7±1223.5	4632.2±1447.4	0.154 (0.26)	3445.2±1443.4	4521.9±1749.6	<0.0001 (0.79)
Central abdominal fat (g)	1477.6±311.4	1662.2±460.2	0.031 (0.58)	1228.5±438.7	1689.4±524.0	<0.0001 (0.98)
Non-abdominal fat (g)	14439.2±5083.8	17603.0±6905.3	0.005 (0.67)	13160.4±4255.3	18 763.8±8789.4	<0.0001 (0.88)
	Type 2 diabetic subjects (n = 82)			*NGT subjects (n = 82)*		
	Gly/Gly (n = 38)	*X/Ser (Gly/Ser+Ser/Ser) (n = 44)*	*P-value* (power)*	*Gly/Gly (n = 48)*	*X/Ser (Gly/Ser+Ser/Ser) (n = 34)*	*P-value* (power)*
Gly482Ser polymorphism						
Total body fat (g) (body fat %)	19706.6±5179.5 (30.7±8.5)	20871.9±6989.5 (31.4±6.1)	0.433 (0.14)	19180.8±9049.5 (29.0±6.4)	19 113.1±6235.8 (31.6±7.7)	0.972 (0.05)
Abdominal fat (g)	4661.2±1229.9	4418.3±1376.6	0.445 (0.14)	3849.9±1726.4	3906.2±1581.2	0.888(0.05)
Central abdominal fat (g)	1643.4±367.5	1534.1±410.1	0.251 (0.25)	1394.0±539.8	1394.9±499.8	0.994 (0.05)
Non-abdominal fat (g)	15045.4±4452.5	16131.0±6331.4	0.411 (0.15)	15336.4±7691.9	15 207.0±5165.7	0.937 (0.05)
	Type 2 diabetic subjects (n = 82)			*NGT subjects (n = 82)*		
	AA (n = 35)	*XG (AG+GG) (n = 47)*	*P-value* (power)*	*AA (n = 43)*	*XG (AG + GG) (n = 39)*	*P-value* (power)*
+A2962G 3′-UTR polymorphism						
Total body fat (g) (body fat %)	20748.3±6260.3 (30.2±7.2)	20155.8±6261.8 (31.1±7.2)	0.708 (0.07)	17982.5±6914.9 (30.8±7.2)	19 415.1±8748.0 (30.5±7.3)	0.457 (0.13)
Abdominal fat (g)	4517.6±1337.2	4529.0±1336.7	0.973 (0.05)	3850.0±1668.0	3840.7±1754.2	0.982 (0.05)
Central abdominal fat (g)	1609.7±443.8	1594.1±369.9	0.878 (0.05)	1399.1±539.9	1379.5±536.4	0.879 (0.05)
Non-abdominal fat (g)	15860.3±6008.2	15626.8±5303.3	0.869 (0.05)	14144.2±5816.6	15 574.4±7271.3	0.374 (0.16)

Data are presented as mean ±s.d. **P*-value adjusted for age, sex and type 2 diabetes.

23285.7±10229.5 (34.1±8.7%) vs GG 16601.0±5177.1 (29.1±6.1%)g, $P<0.0001$), abdominal fat (XA 4521.9±1749.6 vs GG 3445.2±1443.4 g, $P<0.0001$), central abdominal fat (XA 1689.0±524.0 vs GG 1228.5±438.7 g, $P<0.0001$) and non-abdominal fat (XA 18763.8±8789.4 vs GG 13160.4±4255.3 g, $P<0.0001$) were also significantly higher in the subjects with XA genotype compared to those with GG genotype. The *P*-values shown above for the obesity phenotypes are adjusted for age, sex and type 2 diabetes. There was no association between Gly482Ser or +A2962G polymorphisms and any of the body fat measurements made on DXA.

The study subjects with and without the Thr394Thr polymorphism were stratified based on gender and analyzed for the association of the variant with the obesity phenotypes in men and women. In women, total abdominal, visceral, subcutaneous fat, total body fat, abdominal and central abdominal fat were significantly higher in NGT subjects with XA genotype of the Thr394Thr polymorphism compared to those with GG genotype (total abdominal fat: XA 479.3±186.9 vs GG 282.9±98.0 cm^2, $P<0.0001$; visceral fat: XA 137.3±45.8 vs GG 81.0±31.7 cm^2, $P<0.0001$; subcutaneous fat: XA 340.5±174.5 vs GG 201.0±84.9 cm^2, $P=0.001$; abdominal fat: XA 4612.1±2157.1 vs GG 2865.5±914.8 cm^2, $P<0.0001$; and central abdominal fat: XA 1722.9±634.1 vs GG 1037.5±290.5 cm^2, $P<0.0001$). In males, total abdominal, visceral, subcutaneous fat, total body fat, abdominal and central abdominal fat were significantly higher in NGT subjects with XA genotype of the Thr394Thr polymorphism compared to those with GG genotype (total abdominal fat: XA 420.2±150.3 vs GG 282.9±98.0 cm^2, $P=0.038$; visceral fat: XA 180.6±51.5 vs GG 82.9±33.4 cm^2, $P=0.031$; subcutaneous fat: XA 260.1±150.8 vs GG 159.8±65.6 cm^2, $P<0.0001$; abdominal fat: XA 4509.0±2135.6 vs GG 2783.9±934.2 cm^2, $P<0.0001$; and central abdominal fat: XA 1651.5±580.7 vs GG 1133.4±288.2 cm^2, $P=0.002$). Similar analysis was carried out in type 2 diabetic subjects and found that subjects with XA genotype had significantly higher values of fat measures. Hence, in this study, we did not find sex-specific differences in the association of the Thr394Thr polymorphism in the *PPARGC1A* gene with obesity.

Power calculation

We performed an analysis to compute the powers of our *t*-test comparisons based on our sample sizes. With respect to Thr394Thr polymorphism, the power was higher among

NGT subjects (>0.75) compared to type 2 diabetic subjects (Tables 2 and 3). However, the power was low in both NGT and type 2 diabetic subjects with respect to Gly482Ser and +A2962G polymorphisms.

Discussion

In this study, we have evaluated three common polymorphisms of the *PPARGC1A* gene and looked at their association with body fat measured by CT and DXA scans in Asian Indians, a high-risk group for diabetes and coronary artery disease.[31] The results suggest that the Thr394Thr (+1302 G→A) polymorphism of the *PPARGC1A* gene is associated with total abdominal fat, visceral and subcutaneous fat as measured by CT, and total body fat, central abdominal fat and non-abdominal fat as measured by DXA. However, we did not find any gender-specific association of this polymorphism with obesity in contrast to the study in White Europeans.[22] This might reflect ethnic differences in susceptibility to obesity.

In an earlier study, we have shown that the Thr394Thr polymorphism is associated with type 2 diabetes in Asian Indians.[23] This study further confirms this association and suggests, in addition, that this association of the Thr394Thr polymorphism with diabetes may be related to increased body fat. Both NGT and type 2 diabetic subjects with XA genotype have higher visceral fat as measured by CT compared to those with GG genotype. This is further confirmed by the fact that central abdominal fat as measured by DXA was significantly higher in subjects with XA genotype compared to subjects with GG genotype. We have earlier shown that visceral (CT) and central abdominal fat (DXA) are associated with diabetes in our population.[13] However, in addition, subjects with the XA genotype also had increased subcutaneous fat and total body fat showing that the association seems to be with body fat in general and not with any particular fat distribution pattern. The *PPARGC1A* gene is involved in the regulation of energy expenditure, and since the chromosomal location 4p15.1 is known to be associated with abdominal subcutaneous fat as reported in the Quebec family study,[21] it is possible that the association of this polymorphism with body fat seen in this study is a true finding. Moreover, linear regression models of obesity phenotypes controlling for type 2 diabetes and the logistic regression models of diabetes status controlling for measures of obesity revealed that the Thr394Thr polymorphism is associated with obesity and type 2 diabetes independently. There appears to be a stronger association of this polymorphism with fat measures in NGT subjects than type 2 diabetic subjects. One of the explanations for this finding could be that in diabetic individuals, body fat distributions may change because of alterations in diet, exercise[32] and medications. For example, metformin is associated with weight loss and glitazones with weight gain and changes in body fat distribution.[33]

The strong linkage with type 2 diabetes and measures of body fat, found in the chromosomal region where the *PPARGC1A* gene is located suggests that somewhere on this locus a common genetic variant, such as the Thr394Thr polymorphism in the *PPARGC1A* gene, must have a measurable effect on obesity, glucose homeostasis or both. However, it is unclear how a silent polymorphism can affect metabolism and/or alter the risk of obesity. Generally, a polymorphism need not be functionally relevant itself, but can be in complete or near-complete LD with a yet unidentified variant that has functional relevance, for example, in the promoter region. Alternatively, the polymorphism can be in LD with polymorphisms in introns that would destabilize pre-mRNA and result in reduced mRNA levels.[34] As intronic regions of the *PPARGC1A* gene have not yet been systematically screened for SNPs, this scenario remains a possibility. Moreover, a silent polymorphism could also affect translation efficiency by changing the codon preference. Another mechanism could be an effect on splice-enhancer regions within the exon and/or activation of a cryptic splice site.[35,36]

The Gly482Ser and +A2962G polymorphisms have been associated with obesity, type 2 diabetes and insulin resistance in other populations;[22,28] however, we failed to observe an association of these polymorphisms either with the measures of obesity or with type 2 diabetes in our population. The finding in this study is in contrast to the study by Esterbauer *et al*,[22] who had shown a gender-specific association of the +A2962G polymorphism of the *PPARGC1A* gene polymorphism with measures of obesity. While it may be possible that the study was underpowered for these two polymorphisms, this is unlikely, as there was no association of these two polymorphisms with diabetes even in our earlier study based on large numbers.[23] This indeed is one of the limitations of this study. As both CT and DXA are expensive tests and owing to logistics involved in bringing study subjects in for these tests, we had to necessarily restrict the number of subjects in this study. Owing to the polygenic nature of the common forms of type 2 diabetes, future studies should examine the potential interactions of the *PPARGC1A* and other gene polymorphisms implicated in adaptive thermogenesis to see if they have additive or synergistic impact on body fat distribution.

One of the potential concerns of a study such as this could be that the population studied may not be genetically homogeneous, thereby resulting in population stratification, which could affect the analyses and produce false-positive results. However, several studies suggest that any bias from uncontrolled PS is, under most circumstances, only minor. Hence, the findings in this study are not likely to be an artifact of population sub-structuring. Moreover, the fact that the same Thr394Thr polymorphism is associated with both diabetes and body fat in Asian Indians suggests that this could be an important gene related to the metabolic

syndrome in this ethnic group. Indeed, in our earlier study, we showed that this polymorphism was also associated with other features of the metabolic syndrome such as systolic blood pressure and dyslipidemia.[23] More studies are needed to confirm the association of this polymorphism with metabolic syndrome in other populations.

In summary, we report that the Thr394Thr (+1302 G→A) polymorphism is associated with both visceral and subcutaneous fat in Asian Indians. Further studies are needed to investigate the genetic, biochemical and pathophysiological basis of this allelic association and its possible relation to type 2 diabetes.

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