Differential effects of the C1431T and Pro12Ala PPARγ gene variants on plasma lipids and diabetes risk in an Asian population

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Abstract We investigated the association of C1431T and Pro12Ala polymorphisms at the peroxisome proliferator-activated receptor γ (PPARγ) locus with plasma lipids and insulin resistance-related variables, according to diabetes status, in a large and representative Asian population from Singapore consisting of 2,730 Chinese, 740 Malays, and 568 Indians. Moreover, we estimated the diabetes risk and examined gene-nutrient interactions between these variants and the ratio of polyunsaturated fatty acid to saturated fat (SFA) in determining body mass index (BMI) and fasting insulin. We found differential effects of these gene variants. The Pro12Ala polymorphism was more associated with plasma lipids and fasting glucose concentrations, whereas the C1431T polymorphism was related to the risk of diabetes. Carriers of the 12Ala allele had higher HDL-cholesterol than did Pro12Pro homozygotes ($P < 0.05$), and the effect of the 12Ala allele on fasting glucose was modified by diabetes status ($P < 0.001$). After controlling for confounders, carriers of the T allele had decreased risk of diabetes compared with CC homozygotes [odds ratio (OR) 0.73, 95% confidence interval (CI) 0.58–0.93; $P = 0.011$]; this effect was stronger in Indians (OR 0.38, 95% CI 0.15–0.92; $P = 0.032$). For both polymorphisms, normal subjects carrying the less prevalent allele had higher BMI ($P < 0.05$). The PUFA/SFA did not modify the effect of these polymorphisms on BMI or insulin.—Tai, E. S., D. Corella, M. Deurenberg-Yap, X. Adiconis, S. K. Chew, C. E. Tan, and J. M. Ordovas. **Differential effects of the C1431T and Pro12Ala PPARγ gene variants on plasma lipids and diabetes risk in an Asian population. *J. Lipid Res.* 2004.** 45: **674–685.**

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Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear hormone receptor superfamily [reviewed in ref. (1)]. It plays an important role in the differentiation of adipocytes and in the regulation of insulin sensitivity; hence, variation in the PPARγ gene may be a risk factor for the development of diabetes and the metabolic syndrome (2–4). Four PPARγ isoforms have been identified: PPARγ1, PPARγ2, PPARγ3, and PPARγ4, which result from either alternative transcription start sites or alternative splicing (5–7). PPARγ1, PPARγ3, and PPARγ4 proteins are identical and are encoded by exons 1–6, whereas PPARγ2 has 30 additional amino acids at its N terminus encoded by the PPARγ2-specific exon B (5, 6). PPARγ2 is expressed predominantly in adipose tissue, whereas PPARγ1 is expressed in a broad range of tissues (8). Yen et al. (9) in a molecular scanning of the human PPARγ in diabetic Caucasians identified two variants in the coding region of the gene: a silent (C>T) substitution at nucleotide 1431 in the sixth exon, common to PPARγ1 and PPARγ2 proteins, and a Pro12Ala missense mutation occurring in the PPARγ2-specific domain.

Numerous studies have been performed on the association between the Pro12Ala mutation and diabetes or insulin resistance-related variables in Caucasian and Asian populations, with conflicting results (3, 10–14). Although a meta-analysis evaluating 16 published studies involving more than 3,000 individuals reported a significant increase in diabetes risk (1.25-fold) associated with the most common Pro allele (15), subsequent studies failed to confirm this association (16, 17). On the other hand, although it has been postulated that PPARγ may play an im-

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portant role in lipid metabolism, directly or by inducing the transcription of target genes (18, 19), to date, results from studies examining the association between PPARγ2 variants and lipid profiles are scarcer and also inconsistent (3, 13, 20–23). In addition, the prevalence of the Pro12Ala polymorphism varies greatly among populations, being much lower in Asians than in Caucasians (24, 25). Overall, this small prevalence may contribute to the inconsistent results by affecting the statistical power of the comparisons. Furthermore, it has been reported that the degree of glucose tolerance can be a modulator of these associations (26, 27). Thus, our aims were as follows: *1*) to study the association of common variants at the PPARγ locus (C1431T and Pro12Ala polymorphisms) with plasma lipids and insulin resistance-related variables, depending on the degree of glucose tolerance, in a large and representative Asian population from Singapore; *2*) as the Singapore population comprises three ethnic groups (Chinese, Malays, and Indians) that exhibit significantly different incidences of diabetes, our second aim was to estimate the risk of diabetes associated with these polymorphisms; and *3*) considering that a gene-nutrient interaction between the Pro12Ala variant and the ratio of polyunsaturated fatty acid to saturated fat (SFA) in determining body mass index (BMI) and fasting insulin has been described in Caucasians (28), our third objective was to examine this interaction in the Singaporean population.

MATERIALS AND METHODS

Subjects and study design

We have studied 4,038 individuals (1,869 men and 2,169 women) from the 1998 Singapore National Health Survey. The detailed methodology has been described elsewhere (29). Briefly, the survey protocol was based on the World Health Organization (WHO)-recommended model for field surveys of diabetes and other noncommunicable diseases and the WHO MONitor trends in CArdiovascular diseases (MONICA) protocol for population surveys. Initially, 11,200 individuals from addresses representing the house type (a proxy for socioeconomic status) distribution of the entire Singapore housing population were selected from the National Database on Dwellings. A process of disproportionate stratified and systematic sampling was used to select individuals between 18 and 69 years old from this data set with oversampling of the minority groups to ensure that prevalence estimates for the minority groups were reliable and to allow statistical comparison between ethnic groups. The ethnic composition of the sample was 64% Chinese, 21% Malays, and 15% Indians. Every individual has been classified as Chinese, Malay, or Indian depending on self-reported family origin from two generations. Moreover, the possibility of population admixture is slight, because in this country, interethnic marriage is very rare because of the traditional socioeconomic differences among ethnic groups.

In this work, we present data from a random sample of 4,038 individuals (2,730 Chinese, 740 Malays, and 568 Indians) who had complete data for genetic (Pro12Ala and C1431T polymorphisms), clinical, biochemical, and lifestyle (tobacco smoking, alcohol consumption, and physical activity) variables examined. Informed consent was obtained from all participants, and the Ethics Committee of the Ministry of Health of Singapore approved the study.

Data on lifestyle factors were collected using an interviewer-administered questionnaire as previously described (30). The classification used for physical activity participation was adapted from the American College of Sports Medicine's classification. Alcohol intake was assessed using a questionnaire based on the Behavior Risk Factor Surveillance Questionnaire from the Centers for Disease Control and Prevention as previously indicated (29, 30). Daily smokers were defined as those who smoked at least one cigarette per day.

The gene-nutrient interaction study was carried out in a representative subsample. Thus, a validated food frequency questionnaire was used to assess intakes of energy, total fat, and specific fatty acids (31) in a random sample of the participants. Subjects were systematically selected (one in two) to participate in the dietary survey. The questionnaire comprised a food list of 159 individual food items grouped into 23 main food types and 25 subtypes. For each food group, careful consideration was given to ensure that foods from the three ethnic groups were represented. The food composition database at the Ministry of Health in Singapore was used to estimate the nutrient content. This questionnaire was previously validated in the Singaporean population against multiple 24 h recalls as well as urinary N excretion (32). Complete dietary data were available for 2,120 individuals (1,295 Chinese, 451 Malays, and 374 Indians) described in this study.

Clinical and biochemical determinations

Subjects were instructed to fast overnight for at least 10 h. A fasting blood sample was collected, and all subjects except diabetics on medication had a 75 g oral glucose tolerance test. The glucose tolerance of the subjects was determined according to American Diabetes Association recommendations (33) for the diagnosis of diabetes mellitus using both the fasting and the 2 h postchallenge plasma glucose. Three groups of subjects were considered: diabetics, subjects with impaired glucose tolerance (IGT), and normal subjects. Other parameters measured included BMI, waist-to-hip ratio, blood pressure, and plasma lipids (29). All blood specimens for lipids and insulin were collected, centrifuged on site, and sent to the Biochemistry Laboratory of the Department of Pathology at the Singapore General Hospital for analysis on the same day. Serum lipid and glucose concentrations were measured using kits from Boehringer Mannheim Systems (Mannheim, Germany) and read on a BM/Hitachi 747 analyzer (Roche Diagnostics Corp., Indianapolis, IN). Total cholesterol [intra-assay coefficient of variation (CV) 0.8%, interassay CV 1.7%], triglyceride (intra-assay CV 1.5%, interassay CV 1.8%), and glucose (intra-assay CV 0.9%, interassay CV 1.8%) were measured using enzymatic colorimetric assays. HDL-cholesterol (HDL-C; intra-assay CV 2.9%, interassay CV 3.6%) was measured using a homogenous colorimetric assay, whereas LDL-C (intra-assay CV 0.9%, interassay CV 2.0%) was measured using a homogenous turbidimetric assay. Insulin was measured by microparticle enzyme immunoassay methods using an Abbot (Chicago, IL) AxSYM insulin assay (intra-assay CV 4.1%, interassay CV 2.9%).

Genetic analyses

DNA extraction was carried out using QIAamp DNA blood Midi kits (Qiagen, Hilden, Germany) according to the manufacturer's recommended protocol. Subsequently, genotyping was carried out using the ABI Prism SNapShot multiplex system (Applied Biosystems, Foster City, CA). Briefly, DNA fragments containing the single nucleotide polymorphism (SNP) are amplified by PCR, and the amplified products are cleared of unused primers and deoxynucleoside triphosphates (dNTPs) by digestion with exonuclease I and calf alkaline phosphatase (CIP). Then,

an SNP-specific probe is annealed to the cleared PCR product. In the presence of ddNTP fluorescent dyes (each labeled with a different dye) and DNA polymerase, a single base complementary to the polymorphic base in the targeted site of the PCR sample is extended on the 3′ end of the probe. This process is carried out in a thermal cycler for 25–35 cycles to ensure that all targeted bases are extended. Unincorporated ddNTPs are removed by digestion with CIP, and the reaction is denatured and run on the 3100 ABI Genetic Analyzer. The extended and labeled probes are separated based on their sizes and fluorescent colors and then analyzed automatically by the ABI data-collecting program. The Genotyper software is used to analyze and call the genotype of each reaction. The primers and probes that were used for genotyping are as follows. For the exon B Pro12Ala polymorphism, the forward and reverse primers were GGACAGTGCCAGCCAATTCA and CCACGTCCCCAATAGCCGTA, respectively, which yielded a product of 320 bp. The sequence of the probe was GACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGTGGGAGATTCTCCTATTGAC. For the C1431T polymorphism in exon 6, the forward and reverse primers were GCAGGAGCGGGTGAAGACTC and CGCCCAGGTTTGCTGAATGT, respectively, yielding a fragment of 220 bp. The sequence of the probe to detect this SNP was GACTGACTGACTGACTGACTGACTGACTGACTGACTCACCTGCAGTAGCTGCAC. Standard laboratory practices were used to ensure the accuracy of the genotype data. Internal controls and repetitive experiments were used. Any sample that yielded a weak signal was repeated. In addition, 20% of samples were repeated at random to verify reproducibility.

Statistical analyses

Continuous variables were examined for the normality of their distribution. Triglyceride and insulin were significantly skewed, and these variables were logarithmically transformed to improve normality. Statistical analyses with these variables were performed on transformed data. Chi-square tests (Pearson, Fisher exact test, or the Monte Carlo approach) were used to test differences between observed and expected frequencies, assuming Hardy-Weinberg equilibrium, to test linkage disequilibrium (LD), and to test differences in percentages. Haplotypes were estimated by the EH program, which uses the expectation-maximization algorithm to obtain maximum-likelihood estimates of the haplotype frequencies. Pairwise LD coefficients [D and D′ (D/Dmax)] between the PPARγ variants were estimated by the LINKAGE program. The ANOVA procedure was used to compare mean differences for continuous variables among genotypes or among ethnic groups. *P* values for linear trends between categories were calculated. The influence of covariates in the comparison of means was controlled by multiple linear regression analyses. Stratified analyses by ethnic group or by glucose tolerance status (normal subjects, IGT subjects, and diabetics) were also carried out. Lipid concentrations and insulin resistance-related variables were adjusted for ethnic group, gender, age, BMI, tobacco smoking, alcohol intake, exercise, and glucose tolerance status. The homogeneity of allelic effects according to gender, ethnic group, or glucose tolerance status was tested by introducing the corresponding terms of interaction (in a hierarchical manner) in the more parsimonious regression model. Standard regression diagnostic procedures were used to ensure the appropriateness of these models. To test the interaction between fat intake and the PPARγ polymorphisms on BMI or fasting insulin concentration, the PUFA/SFA ratio was calculated. This variable was used as categorical by computing quartiles from the whole population and as a continuous variable. The corresponding regression models with interaction terms for each polymorphism were fitted according to Luan et al. (28). Additional control for ethnicity, smoking, alcohol, exercise, and glucose tolerance status was considered. To estimate the risk of diabetes or IGT associated with the less common allele of each of the PPARγ polymorphisms, the odds ratio (OR) and 95% confidence interval (CI) were computed by logistic regression analysis. Multiple logistic regression models were also fitted to control for the effect of covariates and effect modifiers. All statistical tests were two-tailed, and $P < 0.05$ was considered statistically significant. Statistical analyses were carried out using SPSS version 10.1 (SPSS, Inc., Chicago, IL).

RESULTS

Demographic, biochemical, clinical, and lifestyle characteristics of the 4,038 study subjects by gender and ethnic group are shown in **Table 1**. Statistically significant ethnic differences for BMI, fasting glucose, and plasma lipid profiles in both men and women were observed. These differences persisted even after controlling for age. Likewise, the three ethnic groups exhibited significant differences in lifestyle variables such as tobacco smoking, alcohol consumption, and physical activity. Furthermore, the prevalence of diabetes varied widely among these groups: Indians had the highest prevalence, followed by Malays and Chinese. All of these subjects were genotyped for the C1431T and Pro12Ala polymorphisms at the PPARγ gene. The distribution of the observed genotypes (**Table 2**) did not deviate from the Hardy-Weinberg expectations in any ethnic group for the Pro12Ala ($P = 0.497$, 0.795, and 0.428 for Chinese, Malays, and Indians, respectively) and C1431T ($P = 0.619$, 0.052, and 0.259 for Chinese, Malays, and Indians, respectively) polymorphisms. Statistically significant differences ($P < 0.001$) in the genotype prevalence across the three ethnic groups were detected for both the Pro12Ala and C1431T polymorphisms. A very low allele frequency for the Ala12 allele was found in Malays (0.032) and Chinese (0.037), whereas the higher allele frequency observed in Indians (0.119) was similar to that reported from northern European populations. In contrast, Indians presented the lowest allele frequency for the T variant (0.169) compared with Malays (0.220) and Chinese (0.252). The physical distance between these polymorphisms is 82,432 bp, and the haplotype analysis indicated that these PPARγ variants were strongly associated in Indians ($D' = 0.799$; $P < 0.001$) followed by Malays ($D' = 0.572$; $P < 0.035$) and Chinese ($D' = 0.555$; $P = 0.001$). Because of the small number of homozygous subjects for the less common alleles, heterozygotes and homozygotes were grouped as Ala12 and T carriers. Four pseudohaplotypes were computed (Table 2). Statistically significant differences ($P < 0.001$) in the prevalence of the specific combinations of the PPARγ variants by ethnic group were noted.

To examine the association between the PPARγ gene, plasma lipids, and insulin resistance-related variables depending on glucose tolerance status, men and women were pooled in the analyses after having checked that there was no sex heterogeneity of genotype effects. Three groups by diabetes status were considered: normal sub-

TABLE 1. Demographic, biochemical, clinical, and lifestyle characteristics of the study subjects by ethnic group and gender

Characteristic	Chinese		Malay		Indian		
	Mean	SD	Mean	SD	Mean	SD	*P*
Men							
Age (years)	38.3	12.3	39.6	12.4	40.9	12.1	0.002
BMI (kg/m^2)	23.5	3.7	24.8	4.2	24.6	4.1	<0.001
Fasting glucose (mmol/l)	5.8	1.3	6.0	1.7	6.4	2.3	<0.001
LDL-C (mmol/l)	3.5	0.9	4.0	1.0	3.9	1.1	<0.001
HDL-C (mmol/l)	1.3	0.3	1.1	0.3	1.0	0.3	<0.001
Triglycerides (mmol/l)	1.7	1.2	1.9	1.3	2.1	1.8	<0.001
Systolic blood pressure (mmHg)	125.2	14.9	125.8	18.1	124.0	15.6	0.367
Diastolic blood pressure (mmHg)	77.6	10.7	77.8	12.0	77.5	11.6	0.912
Daily smokers [n, (%)]	290	(23.3)	157	(44.5)	81	(29.7)	<0.001
Nondrinkers [n, (%)]	504	(40.5)	309	(87.5)	136	(49.8)	<0.001
Physical exercise [n, (%)]							0.023
No exercise	586	(47.2)	159	(45.0)	127	(46.5)	
Regular exercise	236	(19.0)	74	(21.0)	74	(27.1)	
Diabetes status [n, (%)]							<0.001
Diabetic subjects	102	(8.2)	44	(12.5)	53	(19.4)	
IGT subjects	146	(11.7)	65	(18.4)	30	(11.0)	
Normal subjects	995	(80.0)	244	(69.1)	190	(69.6)	
Women							
Age (years)	37.9	12.3	38.2	12.6	40.2	12.0	0.013
BMI (kg/m^2)	22.1	3.6	26.2	5.4	25.7	4.9	<0.001
Fasting glucose (mmol/l)	5.5	1.3	6.1	2.5	6.1	2.0	<0.001
LDL-C (mmol/l)	3.3	0.9	3.8	1.1	3.5	0.9	<0.001
HDL-C (mmol/l)	1.6	0.3	1.4	0.3	1.2	0.3	<0.001
Triglycerides (mmol/l)	1.2	0.7	1.4	0.9	1.3	0.7	<0.001
Systolic blood pressure (mmHg)	116.9	16.5	123.9	20.5	118.4	16.8	<0.001
Diastolic blood pressure (mmHg)	70.5	10.7	74.8	12.1	70.1	11.2	<0.001
Daily smokers [n, (%)]	49	(3.3)	15	(3.9)	1	(0.3)	0.064
Nondrinkers [n, (%)]	995	(66.9)	375	(96.9)	244	(82.7)	<0.001
Physical exercise [n, (%)]							0.008
No exercise	987	(66.5)	245	(63.2)	182	(61.9)	
Regular exercise	166	(11.2)	54	(14.0)	55	(18.7)	
Diabetes status [n, (%)]							<0.001
Diabetic subjects	106	(7.1)	60	(15.5)	55	(18.6)	
IGT subjects	182	(12.2)	70	(18.1)	45	(15.3)	
Normal subjects	1199	(80.6)	257	(66.4)	195	(66.1)	

BMI, body mass index; -C, -cholesterol; IGT, impaired glucose tolerance. Numbers of study subjects are as follows: for men, Chinese (n = 1,243), Malay (n = 353), and Indian (n = 273); for women, Chinese (n = 1,487), Malay (n = 387), and Indian (n = 295). *P* values were obtained in the comparison among ethnic groups (ANOVA test for means and Chi-square test for percentages).

jects, IGT subjects, and diabetic subjects. The *P* value for the interaction term between the PPARγ polymorphism and diabetes status was also estimated. Because demographic, anthropometric, and lifestyle variables differed among the ethnic groups, multivariate adjustment by age, gender, ethnic group, BMI, tobacco smoking, alcohol consumption, and physical activity was carried out. In addition, the possible heterogeneity of the effect by ethnic group was tested and the *P* value computed for each category of diabetes status (**Tables 3** and **4** for the Pro12Ala and C1431T polymorphisms, respectively). Except for fasting glucose, homogeneity of the effects of these polymorphisms by diabetes status was found. Moreover, excluding the interaction between ethnicity and the Pro12Ala polymorphism in determining triglycerides in diabetic subjects, no heterogeneity of the associations by ethnic group was noted. For the Pro12Ala polymorphism (Table 3), a statistically significantly higher BMI ($P = 0.037$) was observed in normal subjects carrying the Ala12 allele. In diabetics, the Ala12 allele was associated with statistically higher fasting glucose concentrations ($P = 0.048$). No statistically significant associations with plasma lipids were found for this polymorphism in any of the glucose tolerance strata considered. However, a homogeneous increase in HDL-C concentrations in carriers of the Ala12 allele was observed in every stratum. Thus, the three groups of individuals were pooled and the concentrations of HDL-C in carriers and noncarriers of the Ala12 allele were estimated after multivariate adjustment, including control for diabetes status. Statistically significant differences in HDL-C concentrations were then observed, with carriers of the Ala12 allele having a 3.5% higher mean than Pro12 homozygotes ($P = 0.028$). This difference remained statistically significant ($P = 0.016$) even after additional adjustment for the C1413T polymorphism (**Fig. 1A**). The concomitant decrease in triglycerides associated with the Ala12 allele did not reach statistical significance in the analysis of the whole population either without control ($P = 0.350$) or controlling for the C1413T polymorphism (Fig. 1B). One of the reasons for this lack of association may be the hetero-

TABLE 2. Genotype distribution and allele frequencies of the PPARγ polymorphisms by ethnic group in the Singaporean population

Genotype and Allele	Chinese (n = 2,730) n	Chinese (n = 2,730) %	Malays (n = 740) n	Malays (n = 740) %	Indians (n = 568) n	Indians (n = 568) %	*P*
Pro12Ala							
Pro12Pro	2,533	92.8	693	93.6	443	78.0	<0.001
Pro12Ala	192	7.0	46	6.2	115	20.2	
Ala12Ala	5	0.2	1	0.1	10	1.8	
C1431T exon 6							
CC	1,521	55.7	459	62.0	396	69.7	<0.001
CT	1,040	38.1	236	31.9	152	26.8	
TT	169	6.2	45	6.1	20	3.5	
Combined genotypes							
Pro12Pro and CC	1,471	53.9	448	60.5	377	66.4	<0.001
12Ala carrier and CC	50	1.8	11	1.5	19	3.3	
Pro12Pro and T carrier	1,062	38.9	245	17.8	66	11.6	
12Ala carrier and T carrier	147	5.4	36	4.9	106	18.7	
Allele frequency and (95% CI)							
12Ala allele	0.037 (0.032–0.042)		0.032 (0.023–0.040)		0.119 (0.100–0.139)		<0.001
C1431T allele	0.252 (0.238–0.261)		0.220 (0.199–0.241)		0.169 (0.147–0.191)		<0.001
Pairwise linkage disequilibrium							
D′ and (*P*)	0.555 ($P = 0.001$)		0.572 ($P = 0.035$)		0.799 ($P < 0.001$)		

CI, confidence interval; D′, linkage disequilibrium coefficient (D/Dmax) between the peroxisome proliferator-activated receptor γ (PPARγ) variants. Differences by gender across C1431T and Pro12Ala genotypes and the combined genotypes were nonsignificant by Chi-square test ($P = 0.452$, 0.204, and 0.751, respectively).

geneity of the effect observed for this parameter in diabetic subjects depending on the ethnic group ($P = 0.01$ for the interaction term between ethnicity and the Pro12Ala polymorphism). When the effect was examined by ethnic group after multivariate adjustment, a decrease in triglycerides related to the Ala12 allele was observed only in Chinese diabetic subjects (2.14 ± 0.21 mmol/l vs. 1.36 ± 0.43 mmol/l; $P = 0.047$); no difference was observed in Malays (2.46 ± 0.43 mmol/l vs. 2.35 ± 0.78 mmol/l; $P = 0.890$), and a nonsignificant increase was found in Indians (2.27 ± 0.43 mmol/l vs. 2.72 ± 0.60 mmol/l; $P = 0.093$). By contrast, the C1431T polymorphism (Table 4) was not associated with any lipid variable either in the stratified analysis by glucose tolerance group or in the pooled analysis for the whole population. The only variable that reached statistical significance was BMI in normal subjects, with carriers of the T allele having higher BMI than CC homozygotes ($P = 0.036$). When the combined genotype analysis was carried out by considering the four pseudohaplotype groups described in Table 2, no statistically significant association was found either in the stratified analysis by glucose tolerance group or in the pooled analysis (results not shown).

The association between the PPARγ gene variants and diabetes status was further examined. **Table 5** shows the prevalence of the less common PPARγ gene variant according to diabetes status and ethnic group. We found statistically significant differences in the prevalence of the carriers of the T allele at the C1431T polymorphism de-

TABLE 3. Plasma lipids and insulin resistance-related variables according to the Pro12Ala polymorphism by diabetes status adjusted for age, gender, ethnicity, BMI, tobacco smoking, alcohol consumption, and physical activity

Variable	*P* for Interaction Pro12Ala by Diabetes Status	Normal Subjects: Pro12Pro (n = 2,796) Mean	SE	12Ala Carriers (n = 284) Mean	SE	P^a	P^b	IGT Subjects: Pro12Pro (n = 499) Mean	SE	12Ala Carriers (n = 39) Mean	SE	P^a	P^b	Diabetic Subjects: Pro12Pro (n = 374) Mean	SE	12Ala Carriers (n = 46) Mean	SE	P^a	P^b
Age (years)		35.9	0.2	37.4	0.7	0.098		43.3	0.6	44.9	1.8	0.409		50.5	0.6	49.9	1.6	0.713	
BMI (kg/m^2)	0.873	23.50	0.13	24.12	0.28	0.037	0.324	25.66	0.34	25.78	0.77	0.875	0.715	26.99	0.40	27.48	0.84	0.566	0.693
Fasting glucose (mmol/l)	<0.001	5.37	0.02	5.33	0.03	0.221	0.421	5.68	0.04	5.56	0.99	0.192	0.527	9.21	0.28	10.38	0.59	0.048	0.452
Fasting insulin (mU/l)	0.089	7.16	0.16	6.81	0.36	0.306	0.828	9.31	0.43	9.40	0.96	0.929	0.168	10.78	1.56	7.69	2.90	0.555	0.758
Total cholesterol (mmol/l)	0.615	5.43	0.03	5.45	0.07	0.776	0.472	5.86	0.09	5.80	0.20	0.667	0.324	6.06	0.10	6.29	0.21	0.283	0.423
Triglycerides (mmol/l)	0.982	1.43	0.03	1.36	0.06	0.279	0.798	1.99	0.09	1.93	0.22	0.546	0.168	2.31	0.16	2.10	0.34	0.883	0.017
HDL-C (mmol/l)	0.516	1.31	0.01	1.35	0.02	0.090	0.695	1.24	0.03	1.28	0.06	0.473	0.149	1.13	0.02	1.22	0.05	0.078	0.882
LDL-C (mmol/l)	0.381	3.49	0.03	3.51	0.06	0.641	0.308	3.95	0.08	3.88	0.19	0.720	0.872	4.15	0.09	4.45	0.20	0.121	0.471

[a] *P* values obtained in the comparison between Pro12Pro and carriers of the 12Ala allele after multivariate adjustment (age, gender, ethnicity, BMI, alcohol, tobacco, and physical activity).

[b] *P* values for the interaction term between the Pro12Ala polymorphism and ethnicity in each group of diabetes status.

TABLE 4. Plasma lipid and insulin resistance-related variables according to the C1431T polymorphism by diabetes status adjusted for age, gender, ethnicity, BMI, tobacco smoking, alcohol consumption, and physical activity

Variable	*P* for Interaction C1431T by Diabetes Status	Normal Subjects						IGT Subjects						Diabetic Subjects					
		CC (n = 1,783)		T Carriers (n = 1297)				CC (n = 319)		T Carriers (n = 219)				CC (n = 274)		T Carriers (n = 146)			
		Mean	SE	Mean	SE	P^a	P^b	Mean	SE	Mean	SE	P^a	P^b	Mean	SE	Mean	SE	P^a	P^b
Age (years)		36.6	0.4	36.6	0.4	0.355		44.2	1.1	44.3	1.0	0.719		48.6	1.0	51.8	1.2	0.014	
BMI (kg/m^2)	0.472	23.44	0.14	23.82	0.17	0.036	0.502	25.69	0.37	25.65	0.44	0.934	0.126	26.83	0.41	27.22	0.54	0.469	0.350
Fasting glucose (mmol/l)	0.011	5.35	0.02	5.37	0.02	0.769	0.383	5.70	0.05	5.62	0.06	0.136	0.153	9.39	0.28	9.24	0.39	0.706	0.975
Fasting insulin (mU/l)	0.175	7.17	0.17	7.04	0.21	0.537	0.802	9.48	0.46	9.34	0.56	0.803	0.837	11.07	1.85	9.93	2.19	0.731	0.653
Total cholesterol (mmol/l)	0.965	5.42	0.04	5.47	0.04	0.602	0.651	5.86	0.10	5.78	0.12	0.507	0.637	6.09	0.10	6.14	0.16	0.214	0.072
Triglycerides (mmol/l)	0.702	1.43	0.03	1.42	0.03	0.927	0.327	1.97	0.10	2.07	0.13	0.617	0.719	2.38	0.17	2.20	0.22	0.655	0.535
HDL-C (mmol/l)	0.981	1.32	0.01	1.32	0.02	0.779	0.452	1.24	0.03	1.24	0.04	0.875	0.985	1.14	0.03	1.14	0.03	0.900	0.993
LDL-C (mmol/l)	0.952	3.48	0.03	3.50	0.04	0.546	0.883	3.95	0.09	3.89	0.11	0.609	0.524	4.16	0.10	4.26	0.12	0.408	0.686

[a] *P* values obtained in the comparison between CC individuals and carriers of the T allele after multivariate adjustment (age, gender, ethnicity, BMI, alcohol, tobacco, and physical activity).

[b] *P* values for the interaction term between the C1431T polymorphism and ethnicity in each group of diabetes status.

pending on diabetes status, with a clear trend for the decrease of prevalence of this genetic variant from normal subjects to diabetic subjects (*P* for trend = 0.006). This decreasing trend was mainly found in Indians. Considering the ethnic heterogeneity in the prevalence of diabetes and of the genotype distribution, multivariate adjustments controlling for ethnicity and other potential confounding factors were carried out.

First, two groups of subjects were considered: diabetics and nondiabetics (IGT plus normal subjects), and the risk of diabetes (OR and 95% CI) associated with the less common variant was estimated for the whole population by multiple logistic regression analysis after controlling for ethnicity, sex, age, BMI, tobacco smoking, alcohol consumption, and physical activity. Carriers of the T allele presented a lower risk of diabetes compared with CC homozygotes (OR 0.73, 95% CI 0.58–0.93; $P = 0.011$). Although the direction of this estimation was the same in each of the ethnic groups, the magnitude of the effect was greater and statistically significant only in Indians (**Fig. 2**). The results for the Pro12Ala mutation were more inconsistent, and no statistically significant associations were detected, even when the three ethnic groups were pooled (OR 0.92, 95% CI 0.63–1.35; $P = 0.922$).

As IGT subjects constitute a group intermediate between normal subjects and diabetics, to obtain a better estimation of the risk of diabetes associated with the PPARγ gene variants, IGT subjects were excluded from the analyses and the risk of diabetes was estimated by comparing normal subjects versus diabetics. In addition, the risk of IGT was estimated by excluding diabetic subjects. **Table 6** shows the estimation of the risk of diabetes and of the risk of IGT by logistic regression analysis according to the presence of the Ala12 allele, the T allele, or the specific combination of these gene variants after adjustment for sex, age, BMI, ethnic group, tobacco smoking, alcohol consumption, and physical activity. Although the presence of the Ala12 allele was related to a decreased risk of diabetes, the *P* values did not reach statistical significance in any strata. In the total Singaporean population, the Ala12 allele was significantly associated with a decreased risk of

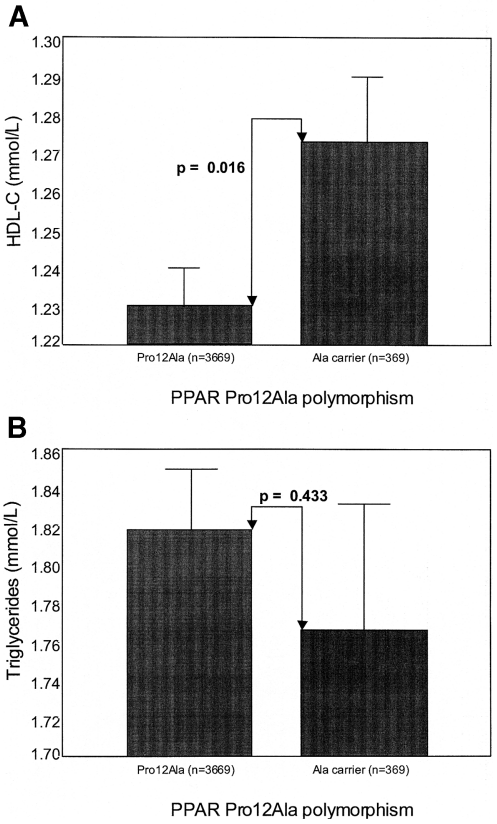

Fig. 1. HDL-cholesterol (HDL-C; A) and triglyceride (B) concentrations in the Singaporean population according to the Pro12Ala polymorphism after adjustment for gender, age, ethnic group, body mass index (BMI), tobacco smoking, alcohol consumption, physical exercise, diabetes status, and the C1431T polymorphism. Values shown are adjusted means ± SEM. p, multivariate adjusted *P* values; PPAR, peroxisome proliferator-activated receptor.

TABLE 5. Prevalence of the less common PPARγ gene variants according to diabetes status and ethnic group in the Singaporean population

Gene Variant	Diabetes Status	Total n	Total %	Chinese n	Chinese %	Malays n	Malays %	Indians n	Indians %
Pro12Ala									
12Ala carriers	Normal	284	7.2	161	7.3	35	7.0	88	22.9
	IGT	39	9.2	18	5.5	6	4.4	15	20.0
	Diabetes	46	11.0	18	8.7	6	5.8	22	20.4
C1431T									
T carriers	Normal	1,297	42.1	976	44.5	193	38.5	128	33.2
	IGT	219	40.7	147	44.8	52	38.5	20	26.7
	Diabetes	146	34.8[a]	86	41.3	36	34.6	24	22.2[b]

[a] *P* for trend = 0.006.
[b] *P* for trend = 0.021.

IGT (OR 0.66, 95% CI 0.58–0.93; $P = 0.026$). Conversely, the T allele was significantly associated with a decreased risk of diabetes, but not IGT, in the whole population. In Indians, both a lower risk of diabetes and a lower risk of IGT were found in carriers of the T allele. In the combined genotype analysis, these alleles did not show an additive effect, with the T allele being associated with a lower risk of diabetes in Indians independent of whether they were carriers of the Ala12 allele or Pro12Pro homozygotes.

Finally, in a random sample of 2,120 Singaporean individuals (1,295 Chinese, 451 Malays, and 374 Indians), dietary intake was measured and the possible gene-nutrient interaction between the PPARγ gene variants and the ratio of PUFA to SFA in determining BMI and fasting insulin were specifically tested. Total fat intake was 26.7, 27.8, and 27.9% of energy intake in Chinese, Malays, and Indians, respectively. PUFA intake was higher in Indians, followed by Chinese and Malays (5.9, 5.3, and 4.8% of energy, respectively). Conversely, SFA intake was higher in Malays, followed by Indians and Chinese (11.8, 11.5, and 10.3%, respectively). Therefore, the ratio of PUFA to SFA was lower in Malays (0.44) than in Chinese (0.55) or Indians (0.55). Quartiles of the PUFA/SFA ratio for the whole population were *1*) <0.33, *2*) from 0.34 to 0.44, *3*) from 0.45 to 0.65, and *4*) >0.65. After adjustment for gender, age, and ethnicity [core model as described by Luan et al. (28)], no statistically significant modification of the effect (P for interaction = 0.099) of the Pro12Ala polymorphism by the PUFA/SFA ratio was found on BMI for the whole Singaporean population (**Fig. 3A**). Further adjustment for diabetes status, tobacco smoking, alcohol consumption, and physical activity did not change the results (P for Pro12Ala by PUFA/SAT interaction = 0.191). Moreover, when the modification of the effect on BMI was tested for the C1431T polymorphism, no statistically significant interaction was found either in the core model (Fig. 3B) or after further adjustment for diabetes status, tobacco smoking, alcohol consumption, and physical activity (P for C1431T polymorphism by PUFA/SAT interaction = 0.872). A similar result of no modification of the effect was obtained when the outcome variable was fasting insulin. No statistically significant interaction terms between the PPARγ polymorphisms and the ratio of PUFA to SFA were found before including or after controlling for BMI in the core model (Fig. 3C, D for the Pro12Ala and C1431T polymorphisms, respectively). Results did not vary after further adjustment for diabetes status, tobacco smoking, alcohol consumption, and physical activity (results not shown). In addition, the variable of PUFA/SAT was considered as continuous, and the corresponding linear regression models were fitted to reproduce the results of Luan et al. (28). No statistically significant interaction terms were found ($P > 0.020$ for all). Furthermore, this potential gene-nutrient interaction was examined by stratifying by ethnic group or by glucose tolerance status. In no situation did the PUFA/SFA ratio in the diet modify the effect of the PPARγ polymorphisms on BMI or fasting insulin (results not shown).

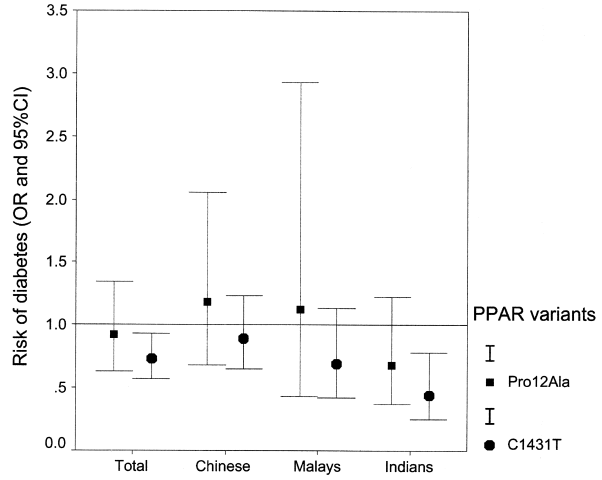

Fig. 2. Risk of diabetes [odds ratio (OR) and 95% confidence interval (CI)] associated with the less common allele of the Pro12Ala and C1431T polymorphisms in the Singaporean population. Homozygotes for the most common allele were considered the reference category. Estimations were determined for the whole population and by ethnic group. Diabetics were compared with nondiabetics (normal subjects plus impaired glucose tolerance subjects), and the OR was adjusted for gender, age, ethnic group, BMI, tobacco smoking, alcohol consumption, and physical exercise.

DISCUSSION

The present report is the first to describe a reduced risk of diabetes in carriers of the T allele of the C1431T polymorphism in exon 6 of the PPARγ gene in Asians. Despite the multiethnic nature of this study, the population stratification cannot be considered an important bias in confounding our results because a careful control by ethnicity, as well as separate analyses by ethnic group, have been conducted (34). In addition, the population admixture was considered small because of the low rates of interethnic marriage in Singapore. Conversely, the Ala12 allele of the Pro12Ala polymorphism was associated with a significantly lower risk of IGT, but it was not associated with dia-

betes risk in Singaporeans. This is consistent with the initial study (3) carried out in Japanese-American subjects, which reported a frequency of the Ala12 allele among IGT subjects intermediate between that of normal and diabetic subjects, with the association of the Pro12Pro genotype with the risk of IGT being near statistical significance (OR 2.62, 95% CI 1.00–6.84; $P = 0.073$). In contrast to our results, the Ala12 allele has also been reported to be protective against type 2 diabetes (T2D) in that (3) and other studies (10, 26, 27), including a meta-analysis (15). Although the initial study (3) reported four times higher risk of diabetes (OR 4.35; $P = 0.028$) in Pro12Pro homozygotes compared with carriers of the Ala12 allele, the magnitude of this association was lower in subsequent studies. Thus, Hara et al. (10) in a case-control study in Japan estimated that carriers of the Ala12 allele had a decreased risk of T2D (OR 0.41, 95% CI 0.22–0.735), in agreement with our results in carriers of the T allele for the C1431T polymorphism among Indians. Moreover, Ek et al. (35) reported in a meta-analysis that the OR of diabetes associated with the Ala12 allele was different in Caucasian (OR 0.85, 95% CI 0.76–0.96) and Asians (OR 0.42, 95% CI 0.26–0.67) populations. However, several other studies did not replicate the protective association with diabetes for the Ala12 allele (11–13, 16, 35). These discrepancies between studies may be related to information bias caused by the degree of glucose tolerance of the subjects, as IGT is an important confounding factor (16). The main advantages of our study in reporting the risk of diabetes associated with each PPARγ variant is that we have stratified by glucose tolerance status. Therefore, we estimated the risk of diabetes both by comparing diabetic subjects with non-

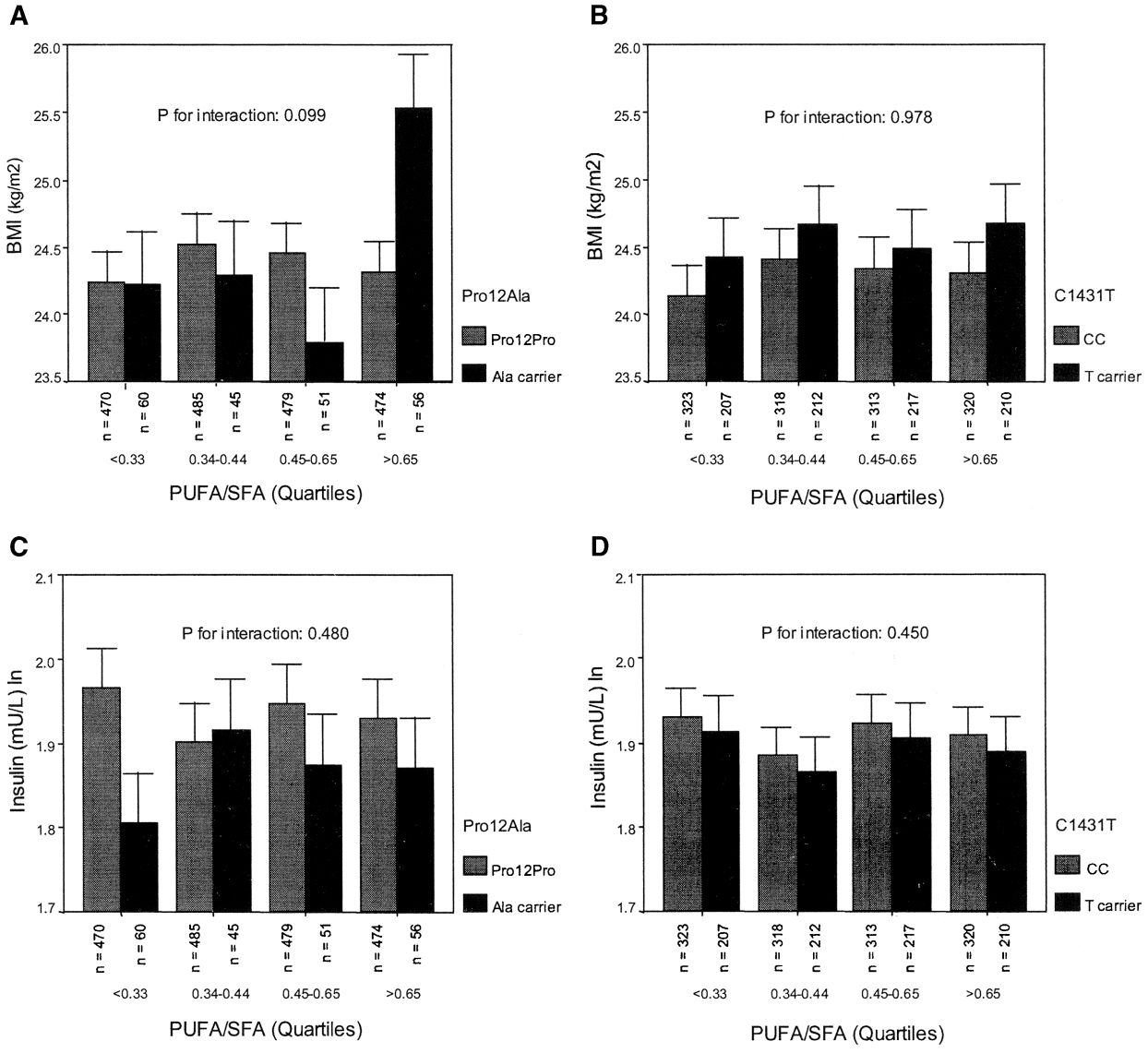

Fig. 3. BMI in the Singaporean population according to the Pro12Ala polymorphism (A) and the C1431T polymorphism (B) and fasting insulin concentrations according to the Pro12Ala polymorphism (C) and the C1431T polymorphism (D) by quartiles of the PUFA-to-saturated fat (SFA) intake ratio in the Singaporean population. Means were adjusted for gender, age, and ethnic group (A and B) and additionally for BMI (C and D). Values shown are adjusted means ± SEM. The *P* value for the interaction term was derived from the corresponding regression model.

TABLE 6. Risk of IGT and risk of diabetes according to the presence of the 12Ala allele, the T allele, or the specific combination of these gene variants and multiple logistic regression analyses in the Singaporean population and stratification by ethnic groups

Gene Variant	Risk	Total				Chinese				Malays				Indians			
		n[a]	OR[b]	95% CI	*P*	n[a]	OR[b]	95% CI	*P*	n[a]	OR[b]	95% CI	*P*	n[a]	OR[b]	95% CI	*P*
Pro12Ala	(Reference)		1				1				1				1		
Pro12Pro	IGT vs. normal	538/3,080	0.66	0.45–0.95	0.026	328/2,194	0.66	0.39–1.15	0.120	135/501	0.56	0.22–1.41	0.222	75/385	0.66	0.34–1.27	0.218
12Ala carriers	Diabetes vs. normal	420/3,080	0.86	0.58–1.27	0.432	208/2,194	0.82	0.61–1.89	0.815	104/501	0.93	0.35–2.61	0.955	108/385	0.64	0.34–1.12	0.159
C1431T exon 6 CC	(Reference)		1				1				1				1		
T carriers	IGT vs. normal	538/3,080	0.92	0.76–1.12	0.416	328/2,194	1.00	0.78–1.30	0.992	135/501	0.95	0.63–1.43	0.803	75/385	0.55	0.30–0.99	0.048
	Diabetes vs. normal	420/3,080	0.73	0.57–0.93	0.011	208/2,194	0.94	0.68–1.30	0.720	104/501	0.67	0.39–1.14	0.142	108/385	0.39	0.21–0.70	0.002
Combined genotypes Pro12Pro/CC	(Reference)		1				1				1				1		
12Ala carrier/CC	IGT vs. normal	538/3,080	0.48	0.20–1.16	0.102	328/2,194	0.34	0.10–1.18	0.088	135/501	0.78	0.14–4.33	0.776	75/385	0.46	0.05–3.96	0.479
	Diabetes vs. normal	420/3,080	1.30	0.62–2.74	0.487	208/2,194	0.84	0.28–2.50	0.755	104/501	1.20	0.17–8.59	0.859	108/385	2.05	0.56–7.54	0.279
Pro12Pro/T carrier	IGT vs. normal	538/3,080	0.96	0.79–1.18	0.689	328/2,194	0.99	0.77–1.28	0.995	135/501	1.03	0.67–1.59	0.879	75/385	0.43	0.17–1.12	0.084
	Diabetes vs. normal	420/3,080	0.76	0.58–0.99	0.048	208/2,194	0.91	0.65–1.29	0.605	104/501	0.67	0.38–1.18	0.164	108/385	0.38	0.15–0.92	0.032
12Ala/T carriers	IGT vs. normal	538/3,080	0.70	0.47–1.05	0.085	328/2,194	0.81	0.44–1.42	0.438	135/501	0.51	0.17–1.54	0.231	75/385	0.61	0.30–1.20	0.151
	Diabetes vs. normal	420/3,080	0.66	0.42–1.04	0.076	208/2,194	1.07	0.54–2.10	0.851	104/501	0.75	0.23–2.42	0.624	108/385	0.42	0.20–0.85	0.017

OR, odds ratio.
[a] Total number of cases included in each logistic regression analysis (number of IGT/number of normal subjects, or number of diabetics/number of normal subjects).
[b] Logistic regression models adjusted for sex, age, BMI, ethnicity, tobacco smoking, alcohol consumption, and physical activity.

diabetics (normal subjects plus IGT subjects) and by comparing diabetic subjects and normal subjects (after exclusion of individuals with IGT). In any case, our findings do not support the hypothesis of a reduced risk of diabetes associated with the Ala12 allele. Our results in a southern Asian population are in agreement with those of Ek et al. (35) in a northern European population. They reported that although the Ala12 allele was not associated with a lower risk of T2D, this allele was associated with improved whole-body insulin sensitivity. These findings are also supported by Frederiksen et al. (36) in the Danish MONICA cohort. After exclusion of T2D subjects, they found that homozygosity for the Ala12 allele conferred a reduction in the risk of the insulin resistance syndrome among nondiabetic subjects.

The controversial findings related to this polymorphism may be attributable to population differences that could be genetic [i.e., the most consistent associations have been found in Japanese subjects (3, 10, 26)] or environmental. Alternatively, the Pro12Ala polymorphism may not be functional, but it may be in LD with the causal mutation. This LD could vary between populations, being higher in those in which the association between the Pro12Ala polymorphism and diabetes is greater. Therefore, in Singaporeans, the C1431T polymorphism would be a better genetic marker than the Pro12Ala of the functional mutation in the PPARγ gene. Even in Singapore, the degree of LD between the C1431T and the Pro12Ala polymorphisms varies between ethnicities, being higher in Indians ($D' = 0.80$) followed by Malays and Chinese ($D' = 0.55$), which supports our hypothesis because the association of the Pro12Ala polymorphism and diabetes was higher in Indians. Another observation supporting the hypothesis that the Pro12Ala polymorphism is a marker for the functional mutation(s) comes from the different prevalence of each of the PPARγ gene variants among the three ethnic groups and its correlation with the incidence of diabetes at the population level. In Singapore, Indians have the highest incidence of diabetes (37) and the lowest prevalence of the T allele, which was a more sensitive marker of the decreased risk of diabetes in our population. By contrast, they present the highest prevalence of the "protective" Ala12 allele.

Additional support for the existence of another causal mutation in the PPARγ gene comes from the work of Muller et al. (38) in Pima Indians. These authors have identified a functional SNP in the promoter region of PPARγ2 in high LD ($D' = 0.98$) with the Pro12Ala polymorphism. This SNP, positioned within a putative E2 box, significantly altered transcriptional activity from a luciferase reporter construct. However, the in vitro functionality of the Pro12Ala mutation has also been demonstrated by Deeb et al. (3) and Masugi et al. (39), who reported decreased transactivation capacity and reduced stimulation of PPARγ target genes for the Ala12 variant compared with the wild-type protein. Conversely, Kolehmainen et al. (40) reported that the Pro12Ala polymorphism has only minor influence on the mRNA expression of PPARγ target genes in adipose tissue of obese subjects.

In summary, the data might indicate that both a relevant functional variant [such as the newly identified PPARγ2 promoter SNP (38) or a mutation in the PPARγ1 protein] and Pro12Ala contribute to the PPARγ2-related phenotypes.

One study has reported opposing effects of the Pro12Ala and C1431T polymorphisms on BMI in Caucasian individuals from the UK (41). However, our results do not support opposite or additive effects of these alleles on the risk of diabetes, BMI, plasma lipids, or insulin resistance-related variables in our population. Despite the significant association of the C1431T polymorphism with a decreased risk of diabetes in our study, we failed to find any significant trait association for this polymorphism when fasting glucose, insulin, or plasma lipid concentration was considered the dependent variable. There are few studies (41–45) analyzing the effects of the C1431T polymorphism, and none of them found significant associations of this gene variant with glucose or lipid-related variables. However, one investigation in Caucasian subjects reported lower concentrations of apolipoprotein B and reduced coronary artery disease risk in carriers of the T allele compared with CC homozygotes (43). Another study in Caucasians reported higher leptin levels in obese subjects bearing the T allele (42), but only one of these studies (45) found a statistically significant association between the C1431T polymorphism and BMI, with subjects bearing the T allele having higher mean BMI. This observation may not be directly related to the C1431T variant but rather the result of the LD between the C1413T and Pro12Ala polymorphisms. In fact, our study shows a statistically significant association between the Pro12Ala polymorphism and BMI in normal subjects, with Ala12 carriers having higher mean BMI. Controversial results have also been obtained for the association between the Pro12Ala polymorphism and BMI, with studies demonstrating greater (16, 45, 46), similar (11, 12, 21, 25), or lower (3) BMI in carriers of the Ala12 allele. It has been proposed that the effect of this polymorphism on BMI would be subtle and greatly related to the degree of insulin sensitivity.

The associations between the Pro12Ala polymorphism and fasting glucose or insulin concentrations have also yielded conflicting results (11–17, 23–27). Overall, the combined evidence suggests that this polymorphism improves insulin sensitivity in nondiabetic subjects (1). However, once diabetes develops, the Ala12 allele may have a deleterious role (8), with carriers of this allele having a lower β-cell function index (26). Our results are in agreement with this notion. We found a statistically significant interaction term between the Pro12Ala polymorphism and diabetes status in determining fasting glucose, with the Ala12 allele being associated with lower plasma glucose concentrations in nondiabetic subjects and with statistically significantly higher concentrations in diabetics.

In terms of lipids, we found a statistically significant association between the Pro12Ala polymorphism and HDL-C concentrations that was not observed for the C1431T polymorphism. Carriers of the Ala12 allele had higher HDL-C concentrations (∼3.5%) than Pro12Pro subjects. We did not detect heterogeneity of this effect by glucose tolerance status, and no interaction by ethnic group was found. Even though we did not observe significantly lower triglyceride concentrations in carriers of the Ala12 allele in the entire Singaporean population, we observed a statistically significant interaction by ethnicity in diabetic subjects. Thus, in Indians with diabetes, the Ala12 allele was associated with statistically significant higher triglyceride concentrations. This association contrasts with the lower triglyceride concentrations associated with the same Ala12 allele in diabetics from Finland (27), in Spanish women from the general population (47), and in Ala12Ala homozygotes in the Danish MONICA cohort (36). However, the vast majority of studies have not found significant associations with plasma lipid concentrations even in obese or in diabetic subjects (10–13, 23, 24, 42, 48).

The association of the PPARγ polymorphism with any lipid or insulin-related traits is troubled by a lack of reproducibility. A prominent role has been suggested for some gene-gene or gene-environment interaction (1). One of the most cited gene-environment interactions with the Pro12Ala polymorphism has been with PUFA intake. Luan et al. (28) reported an interaction between the ratio of dietary PUFA to SFA and this polymorphism on BMI and fasting insulin. According to this interaction, BMI was higher among carriers of the Ala12 allele only when the PUFA/SFA ratio was low, and the opposite effect was noted in the presence of higher PUFA/SFA ratios. The same was demonstrated for fasting insulin (28). However, when we specifically tested this gene-nutrient interaction in the Singaporean population, we were not able to detect it. Our results are in agreement with those of Robitaille et al. (49), who tested this specific interaction on BMI in the Québec Family Study and found no statistical significance. Moreover, their results were in the opposite direction from those of Luan et al. (28). In our study, we also failed to detect a decrease in insulin concentration in subjects bearing the Ala12 allele as the PUFA/SFA ratio increased. The discrepancy between studies may be attributable to multiple factors, including the genetic backgrounds of the populations and additional behavioral factors, such as physical activity (50). However, the large sample size in our study, the similar range of the quartiles of PUFA/SFA intake, and the lack of replication of this gene-nutrient interaction in any of the ethnic or diabetes status groups analyzed argues against the causality of this modification of the effect.

In conclusion, we have found a decreased risk of diabetes in carriers of the T allele of the C1431T polymorphism in exon 6 of the PPARγ gene in the whole Singaporean population that was stronger in Indians. Conversely, this association was not found for the Pro12Ala polymorphism, suggesting that this variant could be a marker for a relevant functional mutation and that the LD between the functional mutation and this common polymorphism varies among populations. The inconsistent findings regarding the association of these polymorphisms with plasma lipids or insulin resistance-related variables can be ex-

plained by their "context-dependent" effect and underscore the need for some standardization in designing and reporting results. Moreover, the lack of reproducibility of the gene-nutrient interaction between the PUFA/SFA ratio on BMI and insulin concentrations reinforces the importance of replication in terms of external validity.

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