

Differential effects of the C1431T and Pro12Ala PPAR γ gene variants on plasma lipids and diabetes risk in an Asian population: The 1998 Singapore National Health Survey

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ABSTRACT

We have investigated the association of C1431T and Pro12Ala polymorphisms at the peroxisome proliferator-activated receptor γ (PPAR γ) locus with plasma lipids and insulin resistance-related variables, according to diabetes status, in a large and representative Asian population from Singapore consisting of 2730 Chinese, 740 Malays and 568 Asian-Indians. Moreover, we estimated the diabetes risk and examined gene-nutrient interactions between these variants and the ratio of polyunsaturated fat (PUFA) to saturated fat (SFA) in determining body mass index (BMI) and fasting insulin. We have found differential effect 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-C than Pro12Pro homozygotes ($P < 0.05$), and the effect of the 12Ala allele on fasting glucose was modified by the diabetes status ($P < 0.001$). After controlling for confounders, carriers of the T allele had decreased risk of diabetes as compared with CC homozygotes (OR:0.73; 95%CI, 0.58-0.93; $P = 0.011$); this effect was stronger in Asian 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.

KEY WORDS: PPAR, polymorphism, lipids, diabetes, insulin, body mass index, fat, diet

INTRODUCTION

Peroxisome proliferator-activated receptor γ (PPAR γ) is a member of the nuclear hormone receptor superfamily (reviewed in [1]). It plays an important role in the differentiation of adipocytes and in the regulation of insulin sensitivity, and hence variation in the PPAR γ gene may be a risk factor for the development of diabetes and the metabolic syndrome (2-4). Four PPAR γ isoforms have been identified: PPAR γ 1, PPAR γ 2, PPAR γ 3 and PPAR γ 4, which result from either alternate transcription start sites or alternate splicing (5-7). PPAR γ 1, PPAR γ 3 and PPAR γ 4 proteins are identical and are encoded by exons 1 to 6, whereas PPAR γ 2 has 30 additional amino acids at its N-terminus, encoded by the PPAR γ 2-specific exon B (5,6). The PPAR γ 2 is expressed predominantly in adipose tissue, whereas PPAR γ 1 is expressed in a broad range of tissues (8). Yen et al (9) in a molecular scanning of the human PPAR γ 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 PPAR γ 1 and PPAR γ 2 proteins, and a Pro12Ala missense mutation occurring in the PPAR γ 2-specific domain.

Numerous studies have been performed on the association between the Pro12Ala and diabetes or insulin resistance-related variables in Caucasian and in Asian populations with conflicting results (3,10-14). Although a meta-analysis evaluating 16 published studies in over 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 such association (16,17). On the other hand, although it has been postulated that PPAR γ may play an important role in lipid metabolism, directly or by inducing the

transcription of target genes (18-19), to date, results from studies examining the association between PPAR γ 2 variants and lipid profile are scarcer and also inconsistent (3,13, 20-23). In addition, prevalence of the Pro12Ala polymorphism greatly varies among populations, being much lower in Asians than in Caucasians (24,25). Overall, this small prevalence may be an important factor contributing 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: 1) To study the association of common variant at the PPAR γ 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 Asian Indians) that exhibit significantly different incidence of diabetes, our second aim was to estimate the risk of diabetes associated with these polymorphisms; 3) Considering that a gene-nutrient interaction between the Pro12Ala variant and the ratio of polyunsaturated fat (PUFA) 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.

MATERIAL AND METHODS

Subjects and study design

We have studied 4038 individuals (1869 men and 2169 women) from the 1998 Singapore National Health Survey. The detailed methodology has been described elsewhere (29). Briefly, the survey protocol was based on the WHO-recommended model for field surveys of diabetes and other non-communicable diseases and the WHO MONICA protocol for population surveys. Initially, 11,200 individuals from addresses representing the house-type (a proxy for socio-economic 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 from this data set with over sampling 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% Asian Indians. Every individual has been classified as Chinese, Malay or Asian Indian depending on its self-reported family origin from two generations. Moreover, the possibility of population admixture is very rare, because in this country, the interethnic marriage is very rare because the traditional socio-economic differences among ethnic groups

In this work we present data from a random sample of 4038 individuals (2730 Chinese, 740 Malays and 568 Asian Indians) who had complete data for genetic (Pro12Ala and C1431T polymorphisms), clinical, biochemical and the life-style variables examined (tobacco smoking, alcohol consumption and physical activity). Informed

consent was obtained from all participants and the Ethics committee of the Ministry of Health of Singapore approved the study.

Data on life-style factors was collected using an interviewer-administered questionnaire as previously described (30). The classification for physical activity participation used 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 sub-sample. 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 (1 in 2) 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 sub-food types. For each food group, careful consideration was given to ensure that foods from the three ethnic groups were represented. The food composition database residing in 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 was available for 2120 individuals (1295 Chinese, 451 Malays and 374 Asian Indians) described in this study.

Clinical and biochemical determinations

Subjects were instructed to fast over-night for at least 10 hours. A fasting blood sample

was collected and all subjects, except diabetics on medication, all subjects had a 75g oral glucose tolerance test (OGTT). The glucose tolerance of the subjects was determined according to the American Diabetes Association (ADA) recommendations (33) for the diagnosis of diabetes mellitus using both the fasting and the 2-hour post challenge plasma glucose. Three groups of subjects were considered: diabetics, subjects with impaired glucose tolerance (IGT), and normal subjects. Other parameters measured included body mass index (BMI), waist-to-hip ratio (WHR) blood pressure (BP) 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 (Boehringer Mannheim, Mannheim, Germany) and read on a BM/Hitachi 747 analyzer (Roche Diagnostics, Corp. Indianapolis, Indiana, USA). Total cholesterol (intra-assay CV 0.8%, inter-assay CV 1.7%), triglyceride (intra-assay CV 1.5%, inter-assay CV 1.8%) and glucose (intra assay CV 0.9%, interassay CV 1.8%) were measured using enzymatic colorimetric assays. HDL-C (intra-assay CV 2.9%, inter-assay CV 3.6%) was measured using a homogenous colorimetric assay whereas LDL cholesterol (intra-assay CV 0.9%, inter-assay CV 2.0%) was measured using a homogenous turbidimetric assay. Insulin was measured by microparticle enzyme immunoassay methods using an Abbot AxSYM (Chicago) insulin assay (intra-assay CV 4.1% and inter-assay CV 2.9%).

Genetic analysis

DNA extraction was carried out using QIAamp DNA blood Midi kits (Qiagen, Hilden Germany) following the manufacturer's recommended protocol. Subsequently,

genotyping was carried out using ABI Prism SNaPShot multiplex system (Applied Biosystems, Foster City, CA). Briefly, DNA fragments containing the single nucleotide polymorphism (SNP)s are amplified by PCR, and the amplified products are cleaned up of unused primers and dNTP by digestion with Exonuclease I and Calf Alkaline Phosphatase (CIP). Then an SNP-specific probe is annealed to the cleaned up PCR product. In presence of ddNTP-fluorescents (each labeled with a different fluorescent 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 insure that all targeted bases are extended. Unincorporated ddNTP 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 being 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, that yielded a product of 320 bp. The sequence of the probe was: GactgactgactgactgactgactgactgactgactgactgTgggagattctctattgac. 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: GactgactgactgactgactgactgactgactgactgactCacctgcagtagctgcac. Standard good laboratory practices were undertaken to assure the accuracy of 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 in order to verify the reproducibility.

Statistical analyses

Continuous variables were examined for normality of their distribution. TG and insulin were significantly skewed, and these variables were logarithmically transformed to improve normality. Statistical analyses with these variables were performed on transformed data. χ^2 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. Pair-wise LD coefficients (D and D' [D/Dmax]) between the PPAR γ 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 the 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. Homogeneity of allelic effects according to gender, ethnic groups or glucose tolerance status was tested by introducing the corresponding terms of interaction (in a hierarchical way) 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 PPAR γ polymorphisms on BMI or fasting insulin concentrations, the PUFA to SFA ratio was calculated. This variable was used as categorical by computing quartiles from the whole population, as well 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 to the less common allele of each one of the PPAR γ 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 a P value <0.05 was considered statistically significant. Statistical analyses were carried out using SPSS v. 10.1 (SPSS Inc, Chicago, Ill).

RESULTS

Demographic, biochemical, clinical and life-style characteristics of the 4038 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 life-style variables such as tobacco smoking, alcohol consumption and physical activity. Furthermore, prevalence of diabetes varied widely among these groups: Asian Indians had the highest prevalence; followed by Malays and Chinese. All these subjects were genotyped for the C1431T and the Pro12Ala polymorphisms at the PPAR γ gene. The distribution of the observed genotypes (**Table 2**) did not deviate from the Hardy-Weinberg expectations in any ethnic group for both the Pro12Ala ($p=0.497$, $p=0.795$ and $p=0.428$; in Chinese, Malays and Asian Indians, respectively) and the C1431T ($p=0.619$, $p=0.052$ and $p=0.259$; in Chinese, Malays and Asian 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 the 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 Asian Indians (0.119) was similar to that reported from Northern European populations. In contrast, Asian Indians presented the lowest allele frequency for the T variant (0.169), as compared with Malays (0.220) and Chinese (0.252). The physical distance between these polymorphisms is 82432 bp, and the haplotype analysis indicated that these PPAR γ variants were strongly associated in Asian Indians (D' : 0.799; $p<0.001$), followed by Malays (D' = 0.572; $p<0.035$) and

Chinese ($D' = 0.555$; $p = 0.001$). Due to the small number of homozygous subjects for the less common alleles, heterozygotes and homozygotes were grouped as Ala12 and T carriers. Four pseudo-haplotypes were computed (Table 2). Statistically significant differences ($p < 0.001$) in the prevalence of the specific combinations of the PPAR γ variants by ethnic group were noted.

To examine the association between the PPAR γ gene, plasma lipids and insulin resistance-related variables depending on the 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 subjects, IGT subjects and diabetic subjects. The P value for the interaction term between the PPAR γ polymorphism and the diabetes status was also estimated. Since demographic, anthropometric and life-style 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 (Table 3 and Table 4 for the Pro12Ala and C1431T polymorphism, 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 significant 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 non-carriers 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 (**Figure 1 A**). The concomitant decrease in triglycerides associate with the Ala12 allele did not reach the statistical significance in the analysis of the whole population, neither without control ($P=0.350$) or controlling for the C1413T polymorphism (**Figure 1 B**). One of the reasons of this lack of association may be the heterogeneity 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 only observed in Chinese diabetic subjects (2.14 ± 0.21 vs 1.36 ± 0.43 mmol/L; $P=0.047$); no differences in Malays (2.46 ± 0.43 vs 2.35 ± 0.78 mmol/L; $P=0.890$), and a nonsignificant increase was found in Asian Indians (2.27 ± 0.43 vs 2.72 ± 0.60) mmol/L; $P=0.093$). By contrast, the C1431T polymorphism (Table 4) was not associated with any lipid variable, neither in the stratified analysis by glucose tolerance group, or in the pooled analysis for the whole population. The only variable that reached the 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 4 pseudo-haplotype groups described in Table 2, no statistically significant association were found neither in the stratified analysis by glucose tolerance group nor in the pooled analysis (results not shown).

The association between the PPAR γ gene variants and diabetes status was further examined. **Table 5** shows prevalence of the less common PPAR γ gene variant, according to the diabetes status and ethnic group. We found statistically significant differences in prevalence of the carriers of the T allele at the C1431T polymorphism depending on the diabetes status, with a clear trend in the decrease of prevalence of this genetic variant from normal subject 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.

Firstly, two groups of subjects were considered: diabetics and non-diabetics (IGT+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 as 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 higher and statistically significant only in Indians (**Figure 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 constitutes a group intermediate between normal subjects and diabetics, in order to obtain a better estimation of the risk of diabetes associated with the PPAR γ 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 the statistical significance in any strata. Whereas, in the total Singaporean population, the Ala12 allele was significantly associated with a decreased risk of IGT (OR=0.66; CI 95%: 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 Asian Indians independently if they were carriers of the Ala12 allele or Pro12Pro homozygotes.

Finally, in a random sample of 2120 Singaporean individuals (1295 Chinese, 451 Malays and 374 Asian Indians), dietary intake was measured, and the possible gene-nutrient interaction between the PPAR γ gene variants and the ratio of PUFA to SFA in

determining BMI and fasting insulin was specifically tested. Total fat intake was 26.7%, 27.8% and 27.9% of energy intake in Chinese, Malays and Asian 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 Asian 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 in Asian Indians (0.55). Quartiles of the PUFA to SFA ratio for the whole population were: 1) <0.33; 2) from 0.34-0.44; 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 (**Figure 3A**). Further adjustment for diabetes status, tobacco smoking, alcohol consumption, and physical activity did not change the results (P for Pro12Ala*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 neither in the core model (**Figure 3B**) nor after further adjustment for diabetes status, tobacco smoking, alcohol consumption, and physical activity (P for C1431T polymorphism*PUFA/SAT interaction: 0.872). Similar results of no modification of the effect were obtained when the outcome variable was fasting insulin. No statistically significant interaction terms between the PPAR γ polymorphisms and the ratio PUFA to SFA were found before including or after controlling for BMI in the core model (**Figures 3C and 3D**, for the Pro12Ala and the C1431T polymorphisms, respectively). Results did not vary after further adjustment for diabetes status, tobacco smoking, alcohol

consumption, and physical activity (not shown). In addition, the variable of PUFA/SAT was considered as continuous, and the corresponding lineal regression models were fitted in order to reproduce the results of Luan et al (28). No statistically significant interaction terms were found ($P > 0.020$ for all of them). Furthermore, this potential gene nutrient interaction was examined by stratifying by ethnic group or by glucose tolerance status. In no situation did the PUFA to SFA ratio in the diet modify the effect of the PPAR γ polymorphisms on BMI or fasting insulin. (Results not shown).

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 PPAR γ 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 has been considered small due to the low rates of inter-ethnic marriage in Singapore. Conversely, the Ala12 allele of the Pro12Ala polymorphism was associated with a significant lower risk of IGT, but it was not associated with diabetes risk in Singaporeans. This is consistent with the initial study (3) carried out in Japanese American subjects that reported a frequency of the Ala12 allele among IGT subjects intermediate between normal and diabetic subjects, with the association of the Pro12Pro genotype with the risk of IGT being near the 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 as 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 of OR: 0.41 (95% CI: 0.22-0.735), more in line with our results in carriers of the T allele for the C1431T polymorphism among Asian Indians. Moreover, Ek et al (35) reported in a meta-analysis that the OR of diabetes associated to the Ala12 allele was found to be different in Caucasians (OR: 0.85; 95%CI: 0.76-0.96)

and in Asians populations (OR: 0.42; 95%CI: 0.26-0.67). 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 due to the degree of the glucose tolerance status 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 PPAR γ variant is that we have stratified by the glucose tolerance status. Therefore, we have estimated both the risk of diabetes by comparing diabetic subjects with non-diabetics (normal subjects + IGT subjects), and the risk of diabetes by comparing diabetic subjects versus 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 Ek et al (35) in a Northern European population. The authors 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 due 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 then be a better genetic marker than the Pro12Ala of the functional mutation in the PPAR γ gene. Even in Singapore, the degree of LD between the C1431T and the Pro12Ala polymorphisms varies between ethnicities, being higher in Asian 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 Asian Indians. Another observation supporting the hypothesis that the Pro12Ala is just marker for the functional mutation (s) comes from the different prevalence of each one of the PPAR γ gene variants among the three ethnic groups and its correlation with the incidence of diabetes at the population level. In Singapore, Asian 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 PPAR γ gene comes from the work by Muller et al (38) in Pima Indians. These authors have identified a functional SNP in the promoter region of the PPAR γ 2 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 lowered transactivation capacity and reduced stimulation of PPAR γ target genes for the Ala12 variant as compared with the wild-type protein. Conversely, Koehmainen et al (40) reported that the Pro12Ala polymorphism has a minor influence on mRNA expression of PPAR γ target genes in

adipose tissue of obese subjects. In summary, the data might indicate that perhaps both a relevant functional variant (as the newly identified PPAR γ 2 promoter SNP [38] or a mutation in the PPAR γ 1 protein) and the Pro12Ala contribute to the PPAR γ 2-related phenotypes.

One study has reported opposing effects of the Pro12Ala and the C1431T polymorphisms on BMI in Caucasian individuals from 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 concentrations were considered the dependent variables. There are few studies (41-45) analyzing the effects of the C1431T polymorphism and none of them has found significant associations of this gene variant with glucose or lipid related variables. However, one investigation in Caucasian subjects has reported lower concentrations of apoB and reduced CAD risk in carriers of the T allele as 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) has found statistically significant association between the C1431T polymorphism and BMI, with subjects bearing the T allele having higher mean. This observation may not be directly related to the C1431T variant but rather the result of the LD between the C1431T and the 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. Controversial results have also

been obtained for the association between the Pro12Ala polymorphism and BMI, with studies demonstrating either greater (16,45,46), no difference (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 non-diabetic subjects (1). However, once diabetes develops, the Ala12 allele may have a deleterious role (8) with carriers of this allele having a lower beta-cell function index (26). Our results are in agreement with this notion. We have 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 non-diabetic subjects, and with statistically significant higher concentrations in diabetics.

In terms of lipids, we have found a statistically significant association between the Pro12Ala polymorphism and HDL-C concentrations that it was not observed for the C1431T polymorphism. Carriers of the Ala12 allele had higher HDL-C concentrations (about 3.5 %) than Pro12Pro subjects. We did not detect heterogeneity of this effect by the 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 on the entire Singaporean population, we observed a statistically significant interaction by ethnicity in diabetic subjects. Thus, in Asian 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 (48) or with the lower concentration of triglycerides seen 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, 49).

Taken together, the association of the PPAR γ polymorphism with any lipid or insulin-related traits is troubled by a lack of reproducibility. In this scenario, it has been suggested a prominent role of some gene-gene or gene-environmental interactions (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 Ala 12 allele only when the PUFA to SFA acids ratio was low and the opposite was noted in the presence of a higher PUFA to SFA ratio. 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 Robitaille et al (50) who tested this specific interaction on BMI in the Québec Family Study and did not find statistical significance. Moreover, their results were in the opposite direction that Luan et al (28). In our study, we also failed to detect a decrease in insulin concentrations in subjects bearing the Ala12 as the PUFA/SFA increased. The discrepancy between studies may be due to multiple factors including the genetic background of the populations and additional

behavioral factors such as physical activity (51). 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 the exon 6 of the PPAR γ in the whole Singaporean population that was stronger in Asian Indians. Conversely, this association was not found for the Pro12Ala polymorphism suggesting that this variant could potentially be a marker for a relevant functional mutation and that the linkage disequilibrium 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 explained by their “context dependency” effect and underscores the need for some standardization in designing and reporting results. Moreover, the lack of reproducibility of the gene-nutrient interaction between the PUFA to SFA ratio on BMI and insulin concentrations, reinforces the importance of replication in terms of external validity.

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

	Men			P*
	Chinese (n=1243)	Malay (n=353)	Indian (n=273)	
	Mean (SD)	Mean (SD)	Mean (SD)	
Age (years)	38.3 (12.3)	39.6 (12.4)	40.9 (12.1)	0.002
Body mass index (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	146 (11.7)	65 (18.4)	30 (11.0)	
Normal subjects	995 (80.0)	244 (69.1)	190 (69.6)	
	Women			
	Chinese (n=1487)	Malay (n=387)	Indian (n=295)	
Age (years)	37.9 (12.3)	38.2 (12.6)	40.2 (12.0)	0.013
Body mass index (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	182 (12.2)	70 (18.1)	45 (15.3)	
Normal subjects	1199 (80.6)	257 (66.4)	195 (66.1)	

*:P value obtained in the comparison among ethnic groups (ANOVA test for means and Chi squared test for percentages)

IGT: Impaired glucose tolerance

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

	Chinese (n=2730)	Malays (n=740)	Indians (n=568)	P
	n (%)	n (%)	n (%)	
Pro12Ala				
Pro12Pro	2533 (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	1521 (55.7)	459 (62.0)	396 (69.7)	<0.001
CT	1040 (38.1)	236 (31.9)	152 (26.8)	
TT	169 (6.2)	45 (6.1)	20 (3.5)	
Combined genotypes				
Pro12Pro and CC	1471 (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	1062 (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
1431T allele	0.252 (0.238-0.261)	0.220 (0.199-0.241)	0.169 (0.147-0.191)	<0.001
Pair-wise linkage disequilibrium				
D' and (P)	0.555 (P=0.001)	0.572 (P=0.035)	0.799 (P<0.001)	

Differences by gender across c1431t and Pro12Ala genotypes and the combined genotypes were nonsignificant
Chi square p values = 0.452, 0.204 and 0.751, respectively.

CI: Confidence interval

D': Linkage disequilibrium coefficient (D/Dmax) between the PPARG variants

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

P interaction Pro12Ala*diabetes status	Normal subjects				IGT				Diabetic subjects			
	Pro12Pro n = 2796	12Ala carriers n = 284	P ¹	P ²	Pro12Pro n = 499	12Ala carriers n = 39	P ¹	P ²	Pro12Pro n = 374	12Ala carriers n = 46	P ¹	P ²
	Mean (SE)	Mean (SE)			Mean (SE)	Mean (SE)			Mean (SE)	Mean (SE)		
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

1: P value obtained in the comparison between Pro12Pro and carriers of the 12Ala allele after multivariate adjustment (age, gender, ethnicity, BMI, alcohol, tobacco and physical activity)

2: 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 and alcohol consumption and physical activity

P interaction C1431T*diabetes status	Normal subjects				IGT				Diabetic subjects			
	CC	T carriers		CC	T carriers		CC	T carriers		P ¹	P ²	
	n = 1783	n = 1297	P ¹	P ²	n = 319	n = 219	P ¹	P ²	n = 274	n = 146	P ¹	P ²
	Mean (SE)	Mean (SE)			Mean (SE)	Mean (SE)			Mean (SE)	Mean (SE)		
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	

1: P value 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)

2: P values for the interaction term between the C1431T polymorphism and ethnicity in each group of diabetes status

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

Gene variant	Diabetes status	Total	Chinese	Malays	Indians
		n (%)	n (%)	n (%)	n (%)
<i>Pro12Ala</i>					
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)
<i>C1431T</i>					
T carriers	Normal	1297 (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

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. Multiple logistic regression analysis in the Singaporean population and stratification by ethnic groups

Gene variant	Risk	Total			Chinese			Malays			Indians		
		n*	OR** (95%CI)	p	n*	OR** (95%CI)	p	n*	OR** (95%CI)	p	n*	OR** (95%CI)	p
Pro12Ala													
Pro12Pro	(Reference)		1			1			1				1
12Ala carriers	IGT vs normal	538/3080	0.66 (0.45-0.95)	0.026	328/2194	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
	Diabetes vs normal	420/3080	0.86 (0.58-1.27)	0.432	208/2194	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	538/3080	0.92 (0.76-1.12)	0.416	328/2194	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	420/3080	0.73 (0.57-0.93)	0.011	208/2194	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	538/3080	0.48 (0.20-1.16)	0.102	328/2194	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	420/3080	1.30 (0.62-2.74)	0.487	208/2194	0.84 (0.28-2.5)	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	538/3080	0.96 (0.79-1.18)	0.689	328/2194	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	420/3080	0.76 (0.58-0.99)	0.048	208/2194	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	538/3080	0.70 (0.47-1.05)	0.085	328/2194	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	420/3080	0.66 (0.42-1.04)	0.076	208/2194	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

*: Total number (n) of cases included in each logistic regression analysis (n of IGT/n of normal subjects, or n of diabetics/n of normal subjects)

** : Logistic regression models adjusted for sex, age, bmi, ethnicity, tobacco smoking, alcohol consumption and physical activity

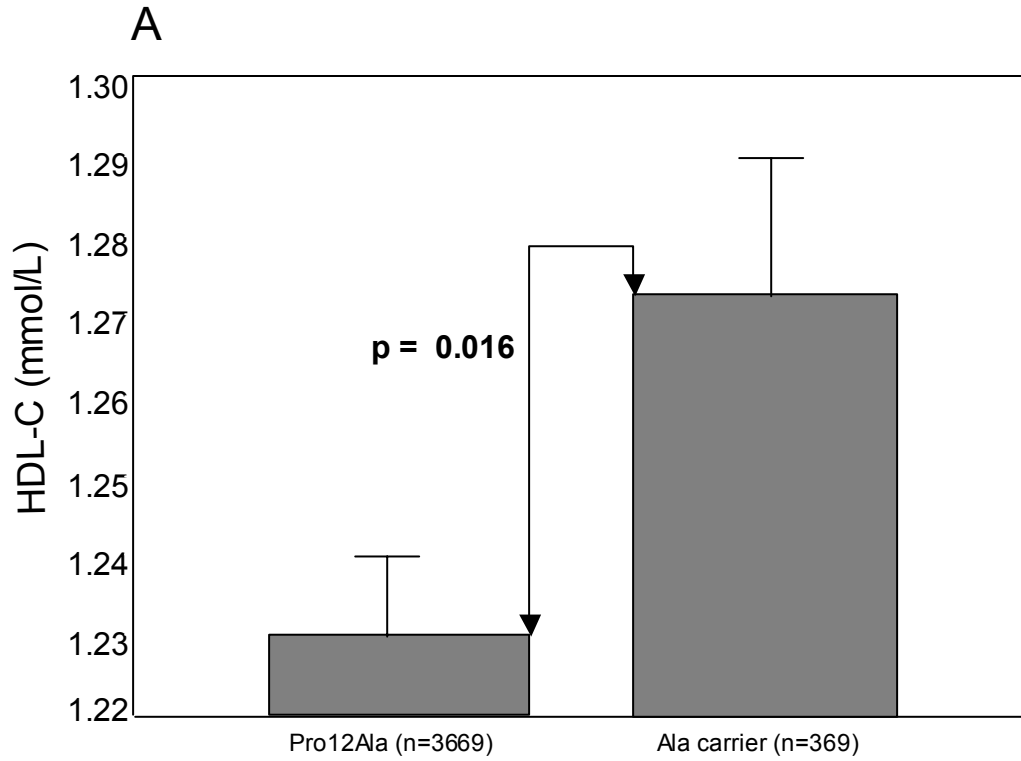
OR: Odds ratio; CI: confidence interval

LEGENDS TO FIGURES

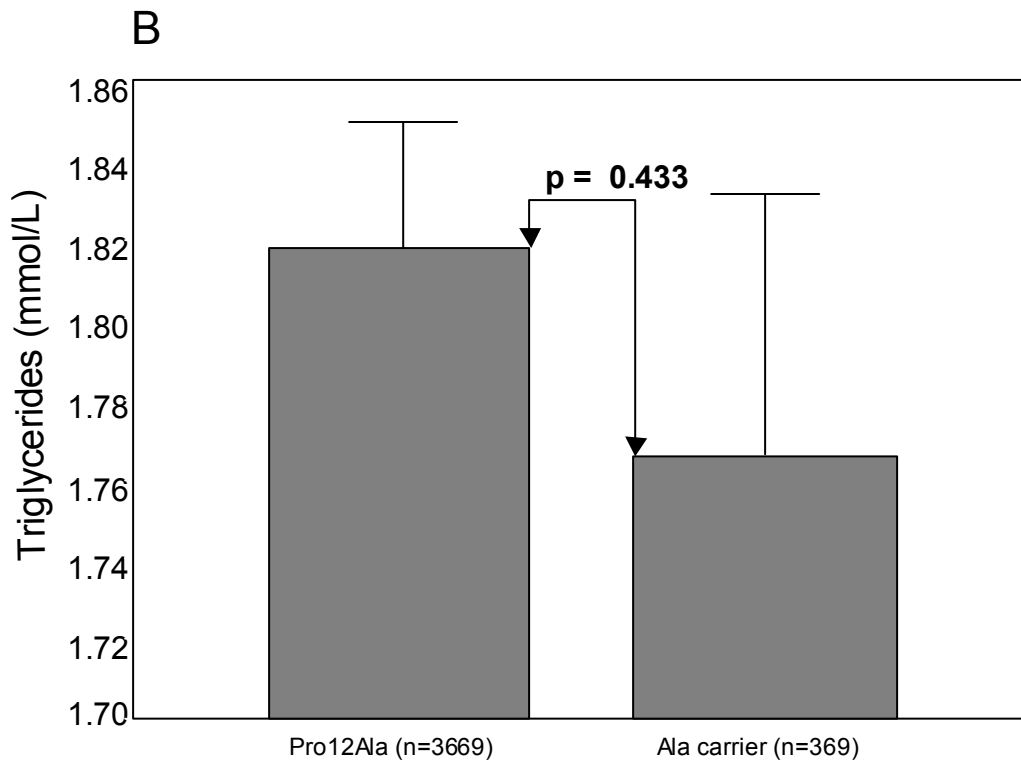
Figure 1: HDL-C (A) and triglycerides concentrations (B) in the Singaporean population according to the Pro12Ala polymorphism after adjustment for gender, age, ethnic group, BMI, tobacco smoking, alcohol consumption, physical exercise, diabetes status and the C143T polymorphism. Error bars: standard errors of means (SEM). Values are adjusted means \pm SEM. P: Multivariate adjusted P value

Figure 2: Risk of diabetes (OR and 95% CI) associated with the less common allele of the Pro12Ala and C1431T polymorphism in the Singaporean population. . Homozygotes for the most common allele were considered the reference category. Estimations for the whole population and by ethnic group. Diabetics were compared with non-diabetics (normal subjects+IGT subjects) and the OR were adjusted for gender, age, ethnic group, BMI, tobacco smoking, alcohol consumption, physical exercise.

Figure 3: BMI in the Singaporean population according to the Pro12Ala polymorphism (A) or the C1431T polymorphism (B) and fasting insulin concentrations according to the Pro12Ala polymorphism (C) or the C1431T polymorphism (D) according to the quartiles of the PUFA to SFA ratio intake in the Singaporean population. Means were adjusted for gender, age, and ethnic group (A and B) and additionally for BMI (C and D). Error bars: standard errors of means (SEM). Values are adjusted means \pm SEM. The P value for the interaction term was derived form the corresponding regression model.

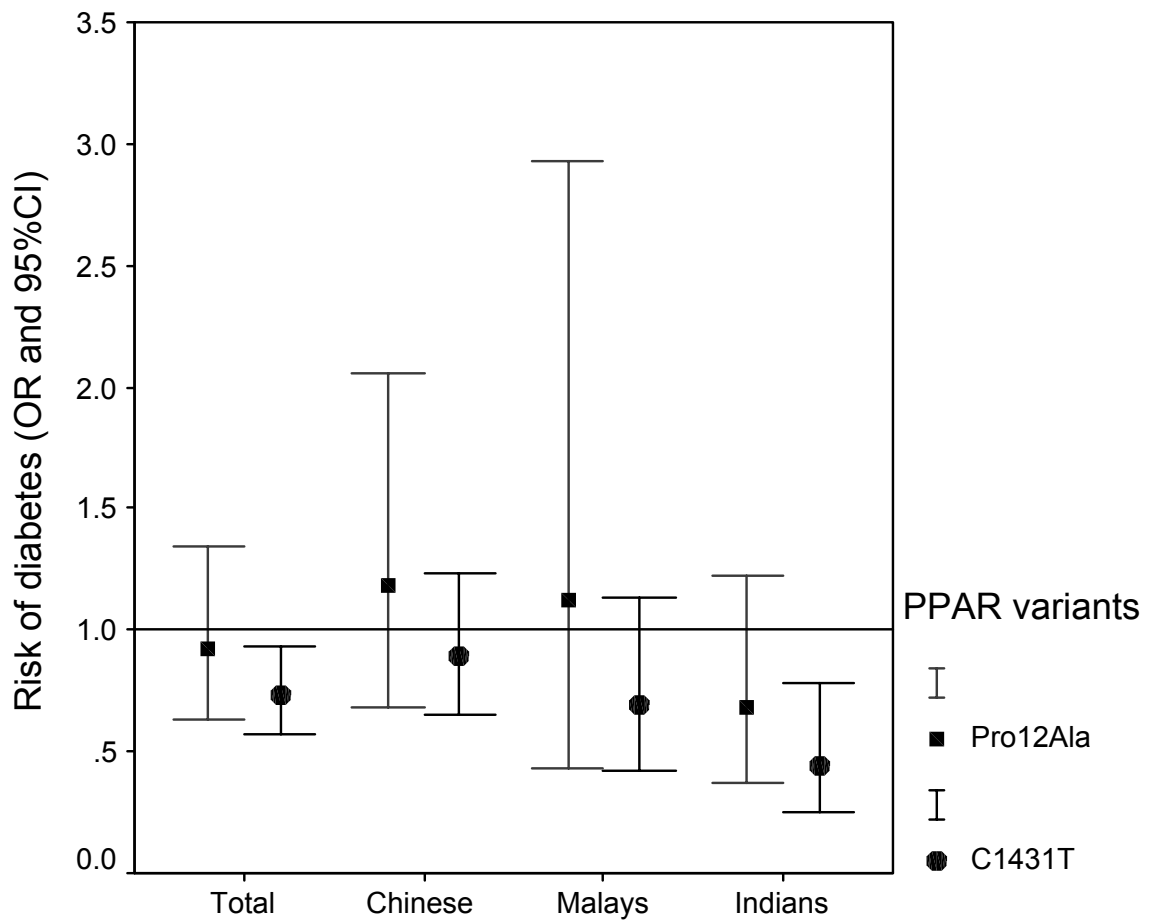


PPAR Pro12Ala polymorphism



PPAR Pro12Ala polymorphism

Fig 2



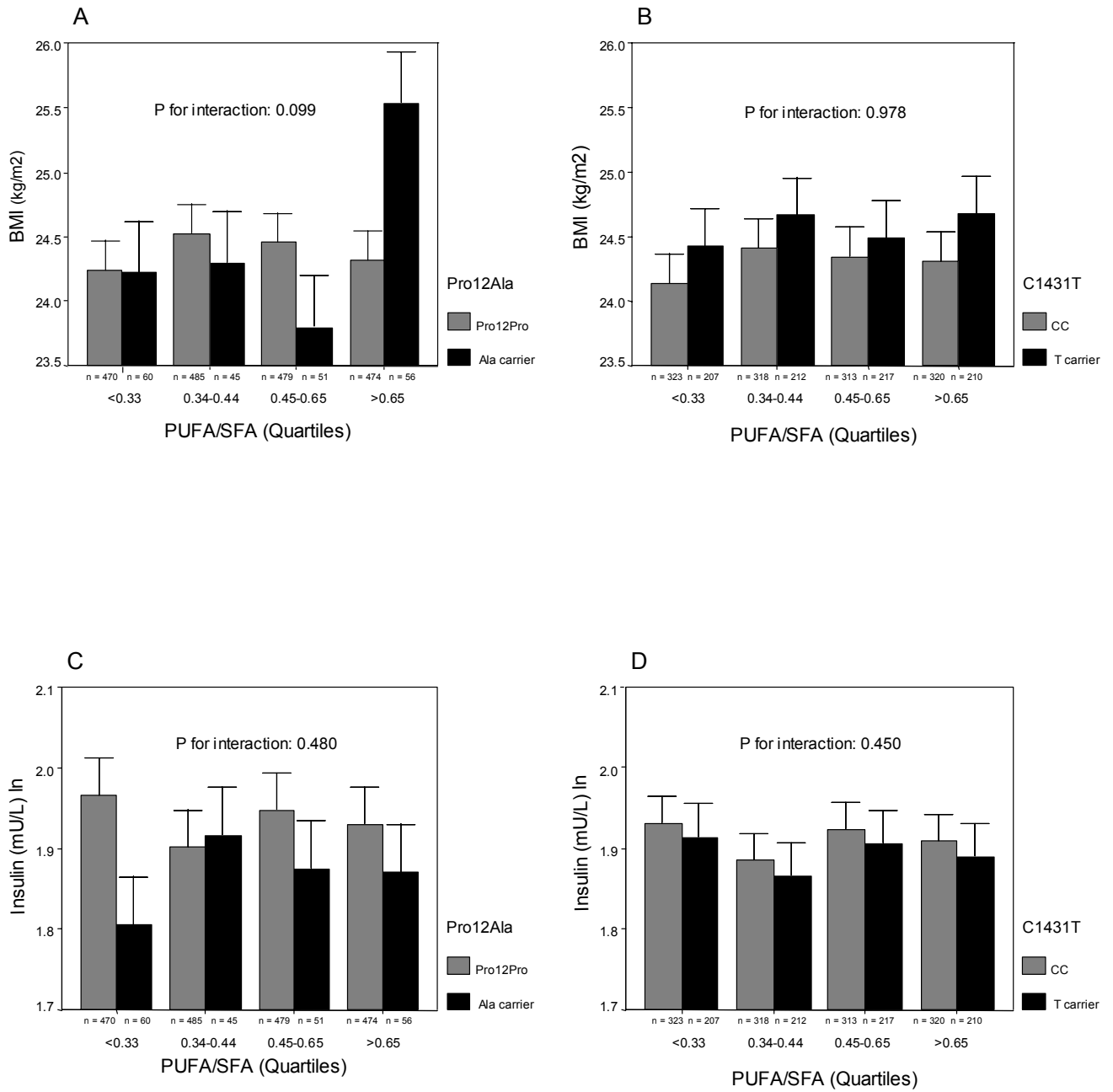


Fig. 3



