

Association of the Pro12Ala, C161-T polymorphisms in the PPAR γ 2 gene with obesity in Han nationality of Yunnan Plateau in Southwest China

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Abstract

Background

Obesity is considered a major public health problem in the world. At present, the association of single nucleotide polymorphisms (SNPS) Pro12Ala and C161-T in peroxisome proliferator-activated receptor gamma 2 (PPAR γ 2) gene with obesity is still controversial. The aim of this study is to evaluate the relationship between the Pro12Ala and C161-T polymorphisms of PPAR γ 2 gene and obesity among Han nationality of Yunnan Plateau in Southwest China.

Methods

The genotypes of 284 extremely overweight patients, 475 obese patients and 759 normal controls from Yongsheng County of Yunnan Province (China) were analyzed. The Pro12Ala and C161-T genotypes of PPAR γ 2 were detected by SNaPshot genotyping assay. The data were analyzed statistically.

Results

The overall prevalence of obesity was 4.99% and the overweight rate was 23.93% among Han nationality in Yongsheng, Yunnan Province. Statistical analysis showed that there were no significant differences in the Pro12Ala genotype, C161-T genotype and the two combined genotypes between the extremely overweight and the control 1, either between the obesity and the control 2 ($P > 0.05$). Further, the alleles of Pro12Ala and C161-T did not exhibit any significant association with extreme overweight or obesity among the complete sample population ($P > 0.05$). However, conditional logistic regression analysis showed that there was a statistically significant association between the two combined genotype of "CC + TT" and the extreme overweight after adjusting for covariates (Calculated with the combined genotype of "CC + CC" as the reference category; $P = 0.014$; OR = 4.04; 95%CI = 1.33–12.33).

Conclusions

Our study indicates that the combined genotypes "CC + TT" of PPAR γ 2 Pro12Ala and C161-T were associated with an increased risk of extreme overweight, however, Pro12Ala and C161-T polymorphisms may not be the main cause of obesity in Han nationality of the Yunnan Plateau in Southwest China.

Background

Obesity is a complex metabolic disorder and has become a major chronic disease affecting all ages and ethnicity, not only in rich countries but also in developing nations(1). It is estimated that there are more than 1.9 billion overweight adults including 650 million obese individuals around the world nowadays(2). Obesity may increase the likelihood of several medical complications, such as cardiovascular disease,

hypertension, type 2 diabetes (T2D), metabolic syndrome, chronic respiratory disease and some forms of cancer(3). The current obesity pandemic is the result of an obesogenic environment that includes high-energy foods and lack of physical activity among those with a genetic susceptibility to obesity(4). In order to reduce the occurrence of obesity, in addition to controlling diet and increasing physical activity, the search for obesity candidate genes has been very active, and more and more genes related to obesity are being discovered. One of these genes is the peroxisome proliferator-activated receptor γ (PPAR γ) gene.

PPAR γ gene, a member of the nuclear hormone receptor superfamily, is a regulator of adipocyte differentiation and energy storage(5). Genes that are essential for adipocyte generation may play an important role in the development of obesity(6) (7). PPAR γ 2 gene polymorphism is closely related to diseases. Many single nucleotide polymorphisms (SNPs) have been found, including Pro12Ala and C161-T, which may be associated with obesity and other diseases(8, 9). It was found that Pro12Ala single nucleotide polymorphism (SNP) regulates the transcriptional activity of the PPAR γ 2 gene, which leads to the substitution of Proline to Alanine on the 12th codon(10). This variant of the PPAR γ 2 gene has also been reported to be associated with obesity, however, inconsistent data were observed in current studies. Among them, some studies have shown that the Pro12Ala variant of the PPAR γ 2 gene is positively associated with obesity and/or obesity-related phenotypes in various populations(11–13), while this variant has produced some negative results in other populations studied(14–16). The C161-T of PPAR γ 2 gene is located in exon 6, and the C161-T polymorphism is the synonym of the 161-bit C expressed as T (CAC→CAT), which was first reported by Meirhaeghe A(17). In recent years, studies have reported that C161-T polymorphism of PPAR γ 2 gene may be associated with chronic diseases such as hypertension, coronary heart disease and obesity(18, 19). At present, there are many studies on the association between PPAR γ 2 gene C161-T polymorphism and chronic diseases, but the results of these studies remain controversial. The association between PPAR γ 2 gene C161-T polymorphism and obesity is rare at home and abroad.

It has been reported that the energy metabolism of obesity may be affected by ambient temperature and altitude. There are three different types of adipose tissue in humans, namely white adipose tissue (WAT), brown adipose tissue (BAT), and beige adipose tissue (BeAT). Both BAT and BeAT are able to increase body temperature while expending energy through the action of uncoupling protein 1 (UCP-1) in mitochondria. Thus, the increase of BAT mass can prevent the development of obesity(20). Cold exposure can increase the expression level of UCP-1 in BAT and BeAT in human(21), which may reduce the incidence of obesity. Studies have shown that adults living at high altitudes have lower rates of obesity, and little is known about the biological mechanisms underlying the association between altitude and obesity(22). Yongsheng County, is located in Yunnan Province of Southwest China, a high latitude area with the average altitude of more than 2500 meters. The annual average temperature is about 12°C, which is a typical plateau cold region. Our previous study showed that the overweight and obesity rates of the Han population in this district were significantly lower than the national average. Therefore, we are interested in the mechanism of obesity under the special geographical environment of cold and high altitude, especially the influence of gene polymorphism on it.

The evidence for assessing the risk of PPAR γ 2 Pro12Ala and C161-T polymorphisms in obesity is currently controversial and needs to be reassessed. There is no related studies been reported in cold and high altitude regions. Therefore, the purpose of this study was to evaluate the association between the PPAR γ 2 Pro12Ala, the C161-T gene polymorphisms and the susceptibility to obesity in adults of Han nationality in the Yunnan Plateau of Southwest China.

Methods

Study population

The present case-control study is part of The China Multi-Ethnic Cohort, Yunnan region, whose baseline survey was carried out between May 2018 and September 2019(23). A total of 1518 subjects were recruited in our study, including 284 extremely overweight subjects ($26.9 \leq \text{BMI} < 28$), 475 obese subjects ($\text{BMI} \geq 28$) and 759 normal controls subjects ($18.5 \leq \text{BMI} < 24$). All participants, aged 30–79 years on the day of the investigation, were from the Chinese Han population in the Yongsheng County of Yunnan Province. We matched the normal weight group (named as control 1) to the extremely overweight group, the normal weight group (named as control 2) to the obese group according to age, gender and town by 1 : 1 ratio. Prior to the survey, all of the participants signed an informed consent form. This study protocol was approved by the Ethics Review Board of Kunming Medical University.

The relevant data about socio-demographic and lifestyle factors of the subjects were collected face to face by questionnaire. Weight, Height, Body Mass Index (BMI), Waist Circumference (WC) and Hip Circumference (HC) were measured by standard methods. Waist-to-hip ratio (WHR) was calculated as a ratio between waist and hips circumference. BMI was calculated as weight in kilograms divided by squared height in meters (kg/m^2). Height in light clothing was determined using a standard steel strip stadiometer to the nearest 0.1 cm. Weight without shoes was measured by digital electronic scale to the nearest 0.1 kg. All subjects were investigated for their systolic blood pressure (SBP) and diastolic blood pressure (DBP) to the nearest 2 mmHg by a trained nurse in a sitting position using an appropriately sized cuff and a standard mercury sphygmomanometer. In addition, after overnight fasting, blood samples for evaluating triglyceride (TG), total serum cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and fasting blood glucose were taken from the anterior cubital vein in the morning. Biochemical markers such as TG, TC and fasting blood glucose were tested by Kunming Jinyu Medical Inspection Institute Co., Ltd.

Genotyping

DNA is extracted from a blood sample using the AxyPreP Blood Genomic DNA Small Volume Kit, according to the manufacturer's instructions. PCR amplification using a perfect match upstream primer [F: 5'-ACGGATTGATC TTTTGCTAGATAGA-3' (Pro12Ala), F: 5'-ACAACCTGCTACAAGCCCTG-3' (C161-T)] and a downstream primer [R:5'-ACATAAATGCCCCACGTCC-3' (Pro12Ala), R:5'-GAAATGTTGGC AGTGGCTCA-3'(C161-T)] was used to create a BstUI restriction site in the PCR product. The quality of the extracted genomic DNA was determined by agarose gel electrophoresis and the content of DNA was

determined by spectrophotometry. All SNP genotyping tests were performed using SNaPshot genotyping test. The PCR products of some samples were randomly selected and sent to Kunming Shuoqing Biotechnology Co., Ltd for genotype sequencing to further verify whether the typing of our SNaPshot method is correct.

Statistical analysis

Data of normal distribution were evaluated by *t* test and analysis of variance (ANOVA). Parameters of skew distribution were analyzed using the Wilcoxon Rank Sum test. The categorical data were analyzed by χ^2 test. Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ^2 test. Conditional Logistic regression was used to adjust the covariates to evaluate the odds ratio (OR) and its 95% confidence interval (*C*). The significance criterion for all tests was set at $P < 0.05$. All data were analyzed using SPSS 23 statistical software.

Results

The subjects in this study were all from the natural population cohort living on the plateau area of Yunnan Province. A total of 10544 subjects were included in the baseline survey, and the obesity rate was 4.99% (526/10544), and the overweight rate was 23.93% (2523/10544). In our study, the distribution of genotype frequency was in line with Hardy-Weinberg balance test. We analyzed polymorphisms of the PPAR γ 2 gene Pro12Ala and C161-T in sample population and their association with the risk of obesity. Table 1 describes the demographic and clinical characteristics of the study population. The results showed that there were significant differences in BMI, WC, HC, WHR, SBP, DBP, TC, TG, LDL and HDL between extreme overweight group and control 1 as well as obese group and control 2 (Table 1, all $P < 0.05$, respectively).

Additionally, the values of fasting glucose were significantly higher in obesity in comparison to control 2 ($P < 0.001$). However, fasting glucose values were similar between extreme overweight and control 1 ($P = 0.052$).

Table 1
Demographic and clinical characteristics of study subjects

| Characteristic | Extreme overweight | Control 1 | Obesity | Control 2 | P value ^a | P value ^b |
|-----------------------------------|---------------------|---------------------|-----------------------|---------------------|----------------------|----------------------|
| | 26.9 ≤ BMI < 27.9 | 18.5 ≤ BMI < 23.9 | BMI ≥ 28 | 18.5 ≤ BMI < 23.9 | | |
| No. of subjects | 284 | 284 | 475 | 475 | | |
| Age (yrs) | 51.71 ± 9.25 | 51.71 ± 9.25 | 51.83 ± 9.28 | 51.83 ± 9.28 | | |
| Gender (Male %) | 36.97 % | 36.97 % | 33.68 % | 33.68 % | | |
| BMI (kg/m ²) | 27.46 ± 0.30 | 21.69 ± 1.56 | 29.70 ± 1.63 | 21.45 ± 1.48 | < 0.001 | < 0.001 |
| Waist circumference (cm) | 89.00 (85.00-92.88) | 74.00 (69.80-79.00) | 93.32 ± 7.24 | 74.42 ± 6.67 | < 0.001 | < 0.001 |
| Hip circumference (cm) | 96.65 (93.58-99.00) | 88.00 (85.00-91.00) | 100.00 (97.00-103.00) | 87.50 (84.50-90.00) | < 0.001 | < 0.001 |
| Waist-to-hip ratio | 0.92 ± 0.06 | 0.85 ± 0.06 | 0.93 ± 0.07 | 0.85 ± 0.06 | < 0.001 | < 0.001 |
| Systolic blood pressure (mmHg) | 131.20 ± 18.52 | 120.19 ± 18.86 | 131.20 ± 18.52 | 120.19 ± 18.86 | < 0.001 | < 0.001 |
| Diastolic blood pressure (mmHg) | 84.83 ± 11.85 | 77.95 ± 11.14 | 84.83 ± 11.85 | 77.95 ± 11.14 | < 0.001 | < 0.001 |
| Fasting glucose (mmol/L) | 5.03 (4.65-5.52) | 4.96 (4.64-5.41) | 5.14 (4.72-5.59) | 4.85 (4.56-5.21) | 0.052 | < 0.001 |
| Total cholesterol (mmol/L) | 5.15 ± 1.00 | 4.88 ± 0.95 | 5.23 ± 1.12 | 4.95 ± 0.91 | 0.001 | < 0.001 |
| Triglyceride (mmol/L) | 2.02 (1.45-2.99) | 1.40 (0.99-1.98) | 2.25 (1.63-3.29) | 1.38 (0.99-1.94) | < 0.001 | < 0.001 |
| High density lipoprotein (mmol/L) | 1.25 (1.05-1.49) | 1.49 (1.29-1.77) | 1.22 ± 0.33 | 1.55 ± 0.41 | < 0.001 | < 0.001 |

Variables with normal distribution are expressed as mean ± SD

Parameters with skewed distribution are expressed as median (P_{25} , P_{75})

^a P values are obtained by comparing the extreme overweight with control 1

^b P values are obtained by comparing the obesity with control 2

| Characteristic | Extreme overweight | Control 1 | Obesity | Control 2 | <i>P</i> value ^a | <i>P</i> value ^b |
|---|--------------------|-------------------|-------------|-------------------|-----------------------------|-----------------------------|
| | 26.9 ≤ BMI < 27.9 | 18.5 ≤ BMI < 23.9 | BMI ≥ 28 | 18.5 ≤ BMI < 23.9 | | |
| Low density lipoprotein (mmol/L) | 3.09 ± 0.92 | 2.91 ± 0.83 | 3.05 ± 0.89 | 2.90 ± 0.79 | 0.014 | 0.003 |
| Variables with normal distribution are expressed as mean ± SD | | | | | | |
| Parameters with skewed distribution are expressed as median (<i>P</i> ₂₅ , <i>P</i> ₇₅) | | | | | | |
| ^a <i>P</i> values are obtained by comparing the extreme overweight with control 1 | | | | | | |
| ^b <i>P</i> values are obtained by comparing the obesity with control 2 | | | | | | |

Table 2 describes the genotype and allele distributions of Pro12Ala, C161-T and the two combined genotypes compared between the case group and the matched-group, respectively. The results showed that in the Pro12Ala polymorphism, the extremely overweight and the control 1 were found three genotypes. These genotypes were CC (Pro/Pro), CG (Pro/Ala) and GG (Ala/Ala). GG genotype was not detected in the obesity and the control 2, which only contained two genotypes, namely CC (Pro/Pro) and CG (Pro/Ala). In the C161-T polymorphism, all four groups showed three genotypes, namely genotypes CC, CT and TT. Statistical analysis of the two SNP loci showed that there were no significant differences in the Pro12Ala genotype, C161-T genotype and the two combined genotypes between the extremely overweight and the control 1, either between the obesity and the control 2 ($P > 0.05$). Further, the alleles of Pro12Ala and C161-T did not exhibit any significant association with extreme overweight or obesity among the complete sample population ($P > 0.05$). Notably, in the two SNP polymorphisms, allele C was the dominant allele in our study and in all previous studies.

Table 2
Distributions of genotypes and alleles between the case and matched-groups

| Groups | Extreme overweight (n = 284) | Control 1 (n = 284) | Obesity (n = 475) | Control 2 (n = 475) | <i>P</i> value ^a | <i>P</i> value ^b |
|------------------------|------------------------------------|------------------------|----------------------|------------------------|--------------------------------|--------------------------------|
| Pro12Ala | | | | | | |
| Genotype * | | | | | 0.762 | 0.916 |
| CC(Pro/Pro) | 264 (0.930) | 256 (0.901) | 424 (0.893) | 422 (0.888) | | |
| CG(Pro/Ala) | 18 (0.063) | 27 (0.095) | 51 (0.107) | 53 (0.112) | | |
| GG(Ala/Ala) | 2 (0.007) | 1 (0.004) | 0 (0.000) | 0 (0.000) | | |
| Allele * | | | | | 0.316 | 0.840 |
| C(Pro) | 546/568 (0.961) | 539/568 (0.949) | 899/950 (0.946) | 897/950 (0.944) | | |
| G(Ala) | 22/568 (0.039) | 29/568 (0.051) | 51/950 (0.054) | 53/950 (0.056) | | |
| C161-T | | | | | | |
| Genotype * | | | | | 0.143 | 0.979 |
| CC | 159 (0.56) | 170 (0.599) | 271 (0.571) | 265 (0.558) | | |
| CT | 109 (0.384) | 104 (0.366) | 179 (0.377) | 183 (0.385) | | |
| TT | 16 (0.056) | 10 (0.035) | 25 (0.053) | 27 (0.057) | | |
| Allele * | | | | | 0.233 | 0.670 |
| C | 427/568 (0.752) | 444/56 (0.782) | 721/950 (0.759) | 713/950 (0.751) | | |
| T | 141/568 (0.248) | 124/56 (0.218) | 229/950 (0.241) | 237/950 (0.250) | | |
| Pro12Ala + C161-T * | | | | | | |
| CC + CC | 155 (0.546) | 166 (0.585) | 260 (0.547) | 256 (0.539) | | |
| CC + CT | 94 (0.331) | 83 (0.292) | 149 (0.314) | 145 (0.305) | | |
| CC + TT | 15 (0.053) | 7 (0.025) | 15 (0.032) | 21 (0.044) | | |
| CG + CC | 4 (0.014) | 4 (0.014) | 11 (0.023) | 9 (0.019) | | |
| CG + CT | 14 (0.049) | 20 (0.070) | 30 (0.063) | 38 (0.080) | | |
| CG + TT | 0 (0.000) | 3 (0.011) | 10 (0.021) | 6 (0.013) | | |

| Groups | Extreme overweight (n = 284) | Control 1 (n = 284) | Obesity (n = 475) | Control 2 (n = 475) | <i>P</i> value ^a | <i>P</i> value ^b |
|--|---------------------------------|------------------------|----------------------|------------------------|-----------------------------|-----------------------------|
| GG + CT | 1 (0.004) | 1 (0.004) | 0 (0.000) | 0 (0.000) | | |
| GG + TT | 1 (0.004) | 0 (0.000) | 0 (0.000) | 0 (0.000) | | |
| * Genotype and allele data are presented as counts and frequency | | | | | | |
| ^a <i>P</i> values are obtained by comparing the extreme overweight with control 1 | | | | | | |
| ^b <i>P</i> values are obtained by comparing the obesity with control 2 | | | | | | |
| <p>Conditional logistic regression analysis was conducted for the extreme overweight and control 1, the obesity group and control 2, respectively. Table 3 describes the odds ratio analysis before and after covariates adjustment. The results of unadjusted analysis for covariates showed that no statistically significant association was found between PPARγ2 Pro12Ala genotypes, C161-T genotypes, combined genotypes and extreme overweight or obesity among our sample population ($P > 0.05$). Interestingly, after adjusting for covariates, there was a statistically significant association between the two combined genotype of "CC + TT" and the extreme overweight (Calculated with the combined genotype of "CC + CC" as the reference category; $P = 0.014$). Compared with subjects who with combined genotype of "CC + CC", the OR for extreme overweight of those with "CC + TT" was 4.04 (95% CI = 1.33–12.33).</p> | | | | | | |

Table 3

Odds ratio analysis before and after adjustment for covariates between the case and matched-groups

| Gene | Genotypes | OR (95 % CI) | P value | Adjusted OR (95 % CI) | P value |
|--|-----------|-------------------|---------|-----------------------|---------|
| (a) Extreme overweight and control 1 | | | | | |
| Pro12Ala | CC | 1.00 (reference) | | 1.00 (reference) | |
| | CG | 0.64 (0.34–1.20) | 0.163 | 0.86 (0.40–1.87) | 0.710 |
| | GG | 2.00 (0.18–22.06) | 0.571 | 1.35 (0.11–16.59) | 0.814 |
| C161-T | CC | 1.00 (reference) | | 1.00 (reference) | |
| | CT | 1.10 (0.78–1.57) | 0.590 | 1.22 (0.79–1.87) | 0.366 |
| | TT | 1.69 (0.74–3.88) | 0.213 | 2.48 (0.95–6.49) | 0.064 |
| Pro12Ala + C161-T | CC + CC | 1.00 (reference) | | 1.00 (reference) | |
| | CC + CT | 1.21 (0.83–1.77) | 0.314 | 1.26 (0.79–1.99) | 0.329 |
| | CC + TT | 2.45 (0.94–6.43) | 0.068 | 4.04 (1.33–12.33) | 0.014 |
| | CG + CC | 1.20 (0.29–4.92) | 0.801 | 0.90 (0.15–5.46) | 0.908 |
| | CG + CT | 0.72 (0.34–1.50) | 0.376 | 1.23 (0.48–3.14) | 0.662 |
| | GG + CT | 1.10 (0.07–17.78) | 0.946 | 0.61 (0.03–11.51) | 0.741 |
| (b) Obesity and control 2 | | | | | |
| Pro12Ala | CC | 1.00 (reference) | | 1.00 (reference) | |
| | CG | 0.96 (0.63–1.45) | 0.833 | 1.32 (0.75–2.31) | 0.338 |
| C161-T | CC | 1.00 (reference) | | 1.00 (reference) | |
| | CT | 0.96 (0.74–1.25) | 0.755 | 0.94 (0.66–1.35) | 0.750 |
| | TT | 0.91 (0.52–1.60) | 0.744 | 0.87 (0.42–1.79) | 0.697 |
| Pro12Ala + C161-T | CC + CC | 1.00 (reference) | | 1.00 (reference) | |
| | CC + CT | 1.03 (0.77–1.38) | 0.832 | 0.96 (0.65–1.43) | 0.856 |
| | CC + TT | 0.69 (0.35–1.37) | 0.287 | 0.66 (0.27–1.60) | 0.360 |
| | CG + CC | 1.20 (0.49–2.93) | 0.694 | 1.82 (0.54–6.16) | 0.333 |
| | CG + CT | 0.79 (0.48–1.31) | 0.361 | 1.04 (0.51–2.14) | 0.908 |
| | CG + TT | 1.64 (0.59–4.55) | 0.343 | 1.72 (0.44–6.64) | 0.435 |
| <i>CI</i> confidence interval; OR odds ratio | | | | | |

Among the extreme overweight and control 1, analysis is obtained after adjustment for covariates including education, smoking status, drinking status, systolic blood pressure, diastolic blood pressure, fasting glucose, total cholesterol, triglyceride, low density lipoprotein and high density lipoprotein

Among the obesity and control 2, analysis is obtained after adjustment for covariates including education, smoking status, drinking status, systolic blood pressure, diastolic blood pressure, fasting glucose, total cholesterol, triglyceride, low density lipoprotein and high density lipoprotein

Discussion

The prevalence of overweight and obesity has risen sharply worldwide over the past few decades, and China reaching a rate of 28.1% of overweight and 5.2% of obesity in 2015(24). The majority of the studies have emphasized the influence of SNP in obesity, especially the polymorphisms of PPAR γ 2 gene Pro12Ala and C161-T. At present, however, there is no report on the correlation between PPAR γ 2 gene polymorphism and obesity of Han nationality in the cold region of Yunnan Plateau. To our knowledge, this is the first study to date to investigate whether the main effects of PPAR γ 2 gene Pro12Ala and C161-T polymorphisms are associated with the risk of obesity in Han ethnic groups in the cold region of Yunnan Plateau.

The PPAR γ 2 Pro12Ala polymorphism has been widely implicated in affecting the risk of obesity (25), although genetic evidence of its effect on obesity is inconsistent. In this study, single locus analysis did not show a significant main effect of PPAR γ 2 Pro12Ala (in genotypic test, or odds ratio analysis) on the risk of obesity in the whole sample population. Our results were consistent with those of several other studies. A previous study by Vaccaro suggested no significant association between PPAR γ Pro12Ala polymorphism and obesity in Italian subjects(26). In addition, Oh EY also presented their own opinion that PPAR γ 2 Pro12Ala may not be a determinant of obesity among Koreans(27). Clement and Ghousaini et al also showed that the PPAR γ 2 Pro12Ala polymorphism was unlikely to influence obesity in French subjects(14, 28). In contrast to our study and the aforementioned studies, the G allele of PPAR γ 2 Pro12Ala has been suggested to be significantly associated with obesity in populations of diverse ethnicities, including Indians, Caucasians in the United States, Finns, Spanish, and Iranians(10, 29–32). The potential reasons for differences between these studies and our results may be the use of diverse phenotype assessments, insufficient sample size, ethnic differences, effects of gender, different study designs, and a lack of adjustment for confounders. The distribution of Pro12Ala polymorphism also varies among different populations. According to the HAPMAP database, the Ala allele frequency ranged between 2.7% and 4.1% in Han population, 0 to 4.4% in Africans, 4.6–16% in European population, 5.3% in Koreans, and 13% in Pakistani population. The previous studies of our research group in Yunnan Province have showed that the G allele frequency of Pro12Ala was 3.17% in Blang people, 2.27% in Wa people, 1.44% in Hani people and 4.66% in Naxi people (data unpublished). In this study, the frequency of G allele in the extreme overweight and control 1 was 3.87% and 5.11%, and in the obese and control 2 was 5.37% and 5.58%, which was close to the Korean, Hui and Naxi ethnic groups, but much lower than

that in Pakistan. It may be influenced by ethnic and environmental factors, thus showing variations between different regions.

Several studies indicated that the distribution of C161-T polymorphism may varies greatly among different countries and ethnical populations. Based on our results, the T allele distribution in the extreme overweight and control 1 was 24.82% and 21.83%, and in the obese and control 2 was 24.11% and 24.95%. Our previous studies in Yunnan Province have found that the T allele frequency of C161-T was 24.50% in Blang nationality, 27.20% in Wa people, 17.35% in Hani people and 18.87% in Naxi people respectively(data unpublished). The carrying rate of T mutation gene in Blang nationality and other domestic studies was basically the same as that in this study (19, 33), however, compared with foreign studies, our results were significantly higher than that of other ethnic groups (16.30% in Caucasians and 13.30% in Japanese women), which may be caused by ethnic differences(34, 35). At present, there are few studies on PPAR γ 2 gene C161-T polymorphism at home and abroad, and some of them are related to chronic diseases such as obesity, diabetes, coronary heart disease and so on. A study has shown that the C161-T variant affects leptin, BMI and blood lipid levels(34). Grygiel-Gorniak et al. reported that the presence of the T allele of the PPAR γ 2 C161-T promoted higher BMI and visceral fat deposition(36). Studies have shown that PPAR γ 2 gene C161-T has the largest CC type BMI, waist circumference and hip circumference, and the smallest TT genotype (37). There are also studies suggesting the opposite conclusion. The Chia PP et al. suggest that C161-T polymorphism is not associated with obesity(38). In our previous studies, we found that the association between C161-T polymorphism and obesity was not found in Yunnan Blang, Wa and Hani nationalities (data unpublished). In our study, no statistical significance was found between the genotype of C161-T polymorphism frequency distribution and the extreme overweight or obesity. We speculated that this might be partly due to the differences in the haplotypes of PPAR γ among different ethnic groups, and Pro12Ala and C161-T were associated with other SNPs in PPAR γ gene. Therefore, other variants around PPAR γ Pro12Ala and C161-T may influence the prevalence of overweight or obesity.

Our study showed that the combined genotypes "CC + TT" of PPAR γ 2 Pro12Ala and C161-T were associated with an increased risk of extreme overweight (OR = 4.04; 95%CI = 1.33–12.33; *P* = 0.014), using "CC + CC" combination genotype as reference category. As far as we know, the present study is the first analysis of the Pro12Ala and C161-T polymorphisms in the PPAR γ 2 gene and obesity.

The study population in the present analysis were all from Yongsheng, Lijiang county of Yunnan province, where belongs to the typical plateau slants cold regions. Special geographical environment may produce certain effect to our study results. As a major regulator of adipocyte development, PPAR γ regulates adipogenesis and adipogenesis pathways in white adipocytes (39), and is required for the differentiation and control of the thermogenesis program in brown or beige adipocytes (40). Exposure to low temperatures modulates the expression of heat shock protein (HSP) in humans and animals, and it has been reported that the HSP20-FBXO4-ubiquitin dependent pathway plays a role in mediating adipocytic function by inhibiting PPAR γ activity of β -adrenergic signaling(41). On this basis, we speculated other possibilities for the results of this study, that is whether the high expression of HSP

under cold exposure inhibits the activity of PPAR γ and reduces its regulatory function on adipocytes, thus covering the possible association between PPAR γ gene polymorphism and obesity, because the study objects are located in a cold region. Of course, this hypothesis needs to be explored in further experimental studies.

One limitation of the study is that the sample was too small to draw firm conclusions. Secondly, the results of our study may not be generalized to other populations, and it is necessary to conduct ethno-matching studies to understand whether such results exist in Han subjects from non-cold areas of the Yunnan Plateau (42). Due to the multifactorial inheritance of obesity, more research must be done to elucidate the role of different genes and SNPs. In future work, it is necessary to conduct large-scale trials to answer the correlation between this SNP and obesity and obesity-related metabolic characteristics.

Conclusion

In conclusion, our study is the first to investigate whether the main effects of Pro12Ala and C161-T polymorphisms of PPAR γ 2 genes are related to the risk of obesity in Han nationality in the cold region of Yunnan Plateau. The results suggest that the combined genotypes "CC + TT" of PPAR γ 2 Pro12Ala and C161-T were associated with an increased risk of extreme overweight, however, Pro12Ala and C161-T polymorphisms may not be the main cause of obesity in Han nationality in the colder areas of the Yunnan Plateau. This may be due to the complexity of obesity, which needs to be further studied.

Abbreviations

BAT
Brown adipose tissue
BeAT
Beige adipose tissue
BMI
Body mass index
CI
Confidence interval
DNA
Deoxyribonucleic acid
DBP
Diastolic blood pressure
HDL
High-density lipoprotein
HC
Hip circumference
LDL
Low-density lipoprotein

OR
Odds ratio
PPAR
Peroxisome proliferators activated receptors
PPAR γ
peroxisome proliferator-activated receptor gamma
PPAR γ 2
Peroxisome proliferator-activated receptor gamma 2
PCR
Polymerase chain reaction
SNP
Single nucleotide polymorphism
SBP
Systolic blood pressure
TC
Total serum cholesterol
TG
Triglyceride
UCP-1
Uncoupling protein 1
WAT
White adipose tissue
WC
Waist circumference
WHR
Waist-to-hip ratio

Declarations

Ethics approval and consent to participate

This case-control study, data analysis and our manuscript were scientifically and ethically approved by the the Ethics Review Board of Kunming Medical University. Each participant with a unique code for individual differentiation provided a written informed consent before enrolment.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this study are included in this article and its Additional file 1.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TZ and JY designed the research and contributed to the interpretation of the data; TZ, NZ analyzed the data and wrote the paper; YG, FM, YQ and GC conducted the research; QM involved in defining variables and developing analysis plan; YF revised the manuscript. All authors read and approved the final manuscript, and they ensured the accuracy and integrity of this article.

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