The Difference of Mutation in the Peroxisome Proliferator Activated Receptor $\gamma_2$ Gene among People at High Altitudes and Low Altitudes in Bolivia

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Abstract: Peroxisome proliferator activated receptor (PPAR) $\gamma$ is present in two isoforms generated by alternative splicing, PPAR $\gamma_1$ and PPAR $\gamma_2$. A Pro12Ala polymorphism in human PPAR $\gamma_2$ moderately reduces its transcriptional activity, and thus PPAR $\gamma_2$ is thought to be a promising candidate gene for several human disorders, including obesity and type 2 diabetes mellitus. In this report, we examined the polymorphism of the PPAR $\gamma_2$ gene in people at high and low altitudes in Bolivia, and found a significant difference in the frequency of Ala carriers (Pro/Ala and Ala/Ala) between 153 native high-altitude Bolivian subjects (64.1%) and 288 low-altitude Bolivian subjects (37.9%). The frequency of this Ala allele in Bolivian subjects was fairly higher than that in other ethnic groups. As body mass index, however, was not associated with Pro12Ala polymorphism of the PPAR $\gamma_2$ gene among either the high altitude Bolivians or low altitude Bolivians, Pro12Ala polymorphism of the gene has little relationship to obesity in Bolivians.

Key words: PPAR $\gamma_2$, thrifty gene, gene polymorphism, obesity.

(Received 16 May 2005, accepted 4 July 2005)

Introduction

Peroxisome proliferator activated receptor (PPAR) $\gamma$ is a nuclear receptor and a ligand-activated transcription factor. It functions as a heterodimer with a retinoid X receptor (RXR), which regulates adipocyte differentiation, expression of adipocyte-specific genes [1–3], and possibly lipid metabolism and insulin sensitivity. PPAR $\gamma$ is present in two isoforms generated by alternative splicing, PPAR $\gamma_1$ and PPAR $\gamma_2$ [4]. PPAR $\gamma_2$ has an additional 28 amino acids at its amino terminus, which renders its ligand-independent activation domain 5–10 fold more effective than that of PPAR $\gamma_1$. A Pro12Ala polymorphism has been detected in the human PPAR $\gamma_2$ gene [5]. The polymorphism in human PPAR $\gamma_2$ moderately reduces its transcriptional activity, and thus PPAR $\gamma_2$ is thought to be a promis-
ing candidate gene for several human disorders, including obesity and type 2 diabetes melilitus. A recent report also showed that the variant Ala12 allele was associated with lower body mass index and improved insulin sensitivity in middle-aged and elderly Finns [6].

The polymorphism of β 3-adrenergic receptor (β 3-AR), which is viewed as a thrifty gene, was demonstrated to have an association with obesity, as well as PPAR γ [7−9]. We previously studied the frequency of Trp 64 Arg polymorphism of the β 3-AR gene among Bolivian people living in the rural areas of high (about 4,000 meters above sea level) and low (about 300 meters above sea level) altitudes to investigate whether population differences in food and/or lifestyle could affect the distribution frequencies of polymorphism in the gene for β 3-AR and we discussed the relationship between the polymorphism of β 3-AR gene and obesity [10]. In rural Andean areas at high altitude, food resources are very limited due to scarce agricultural land of low productivity. This situation contrasts with that at low altitudes in Bolivia, in which people enjoy a more abundant supply of food with the hot and humid weather. A previous study showed that hematocrit, hemoglobin concentration, and Na⁺ and K⁺ excretion into urine are higher among those living at high altitude compared with their counterparts living at low altitude [11]. We found that the percentage of fat to the total body weight is slightly higher in the low-altitude people than the high-altitude people, but no substantial differences in their body mass index or blood pressure were detected. Our previous data indicated that the altitude-related lifestyle of a population had little influence on the frequency of Trp 64 Arg polymorphism and obesity in Bolivian natives [10]. However, we tried to continue investigating whether the long-lasting difference in the lifestyle between the native highlanders and lowlanders in Bolivia had affected the population frequency of polymorphism on other thrifty genes, such as PPAR γ 2. Thus, we examined the polymorphism of the PPAR γ 2 gene in people at high altitude and low altitude in Bolivia, and discussed the association of the difference in lifestyle with the polymorphism of the gene. We also compared the gene polymorphism of Bolivians with that of other ethnic groups.

**Materials and Methods**

**Blood samples**

A total of 441 Bolivian subjects participated in this study: 288 (149 men and 139 women) lived at low altitude and 153 (77 men and 76 women) at high altitude. Blood samples from the Bolivian subjects were obtained during the field studies in 1988−1989. All procedures of the studies were reviewed and approved by the Ethics Committee of the Faculty of Medicine, University of Tokyo (the former affiliation of H. K.). The study on this DNA analysis using thus obtained blood samples was also reviewed and approved by the Ethical Committee of the Research, University of Occupational and Environmental Health, Japan.
Analysis for the point mutation in PPAR γ2 (Pro12Ala)

The polymerase chain reaction (PCR) of PPAR γ2 was carried out in a volume of 30 μl containing 100 ng of genomic DNA from leukocytes; 25 pmol each of the primers (upstream ATG TAC CAA GTC TTG CCA AAG CAG, downstream GCC GTA TCT GGA AGG AAC TTT ACC); 200 μM each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate and deoxythymidine triphosphate; 1.5 mM MgCl2; 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 0.1 percent Triton X-100; 10 percent Glycerol; and 0.5 unit of Taq polymerase (Toyobo Co., Ltd.). The PCR reactions began with denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 53°C for 90 seconds, extension at 72°C for 90 seconds, with a final extension at 72°C for 10 minutes.

The amplified PCR products (350 base pair(bp)) were digested with 0.5 unit of Hga I (BioLabs), a restriction enzyme specific for the sequence GACGC (N)₃, at 37°C for two hours. The digested samples were separated by electrophoresis through a 3 percent agarose gel and visualized by staining with ethidium bromide. The digestion of the 350 bp PCR products with the Hga I produced fragments of the following size: normal homozygote (Pro/Pro type), 350 bp; Heterozygote (Pro/Ala), 350 bp, 286 bp and 64 bp; Homozygote (Ala/Ala), 286 bp and 64 bp.

Statistical analysis

In order to study the association of variables with genotype, chi-square tests were performed.

**Results and Discussion**

We reported previously that the Trp 64 Arg polymorphism of the β 3-AR gene showed no significant difference between the Bolivian subjects living at low and high altitudes and had little relationship with obesity in Bolivian people [10], although a meta-analysis indicated an association of BMI with the polymorphism of the β 3-AR gene in a Japanese population [12]. On the other hand, the studies of Pro12Ala polymorphism in the PPAR γ2 gene among native Bolivian subjects living in different rural areas (Table 1) showed that there was a significant difference ($\chi^2=27.5$, $P<0.001$) in the frequency of Ala carriers (Pro/Ala and Ala/Ala) between the subjects living at high (64.1%) and low (37.9%) altitudes. The frequency (32%) of the Ala allele in Bolivian high-altitude subjects, as calculated from gene frequencies, was higher than that (21%) of Bolivian low-altitude subjects ($\chi^2=14.7$, $P<0.001$), and was also remarkably higher than that of other ethnic groups, as listed in Table 1: Caucasian Americans (12%), Mexican Americans (10%), W. Samoans (8%), African Americans (3%), Nauruans (2%) and Chinese (1%) [13]. A statistical difference was also found in the frequency of the Ala12 allele between low-altitude Bolivians and Mexican...
Table 1. The frequencies of the Pro12 and Ala12 alleles of PPAR γ gene for high-altitude subjects (n = 153) and low-altitude subjects (n = 288) in Bolivia

<table>
<thead>
<tr>
<th>Genotype</th>
<th>High-altitude</th>
<th>Caucasian</th>
<th>Mexican</th>
<th>W. Samoans</th>
<th>African</th>
<th>Nauruans</th>
<th>Chinese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro/Pro</td>
<td>55 (35.9)</td>
<td>77 (47.4)</td>
<td>58 (36.0)</td>
<td>80 (94.6)</td>
<td>92 (92.0)</td>
<td>96 (96.0)</td>
<td>98 (98.0)</td>
</tr>
<tr>
<td>Pro/Ala</td>
<td>97 (63.4)</td>
<td>21 (12.2)</td>
<td>18 (11.2)</td>
<td>14 (14.8)</td>
<td>5 (5.0)</td>
<td>4 (4.0)</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Ala/Ala</td>
<td>10 (7.7)</td>
<td>14 (8.6)</td>
<td>10 (6.4)</td>
<td>6 (6.6)</td>
<td>4 (4.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>153 (100)</td>
<td>26 (16.5)</td>
<td>36 (22.6)</td>
<td>12 (7.2)</td>
<td>53 (39.0)</td>
<td>23 (17.0)</td>
<td>50 (17.0)</td>
</tr>
</tbody>
</table>

Superscript number 1 denotes the reference number
*1: There was a significant difference between high-altitude Bolivians and the indicated ethnic groups (P < 0.05)
*2: There was a significant difference between low-altitude Bolivians and the indicated ethnic groups (P < 0.05)
( ): %

Table 2. Body mass index (BMI) and PPAR γ2 genotypes in Bolivia

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Overweight (BMI ≥ 25)</th>
<th>Non-obese (25 &gt; BMI ≥ 18.5)</th>
<th>Lean (18.5 &gt; BMI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>53 (66)</td>
<td>120 (70)</td>
<td>34 (63)</td>
</tr>
<tr>
<td>Ala</td>
<td>27 (34)</td>
<td>52 (30)</td>
<td>20 (37)</td>
</tr>
<tr>
<td>Total</td>
<td>80 (100)</td>
<td>172 (100)</td>
<td>54 (100)</td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>68 (76)</td>
<td>220 (81)</td>
<td>169 (79)</td>
</tr>
<tr>
<td>Ala</td>
<td>22 (24)</td>
<td>52 (19)</td>
<td>45 (21)</td>
</tr>
<tr>
<td>Total</td>
<td>90 (100)</td>
<td>272 (100)</td>
<td>214 (100)</td>
</tr>
</tbody>
</table>

Americans, African Americans, Nauruans and Chinese. The long-lasting difference in lifestyle between the high- and low-altitude Bolivians may have an influence on the different frequencies of Ala allele, however, the reason why the frequencies of the Ala allele in Bolivians were so high compared with other ethnic groups is not clear. Table 2 shows the PPAR γ2 genotype of high- and low-altitude Bolivian subjects which was categorized according to body mass index (BMI) based on criteria of the World Health Organization [14]. There was no statistical difference in the frequencies of Ala carriers among the overweight, non-obese and lean for either the high- (χ² = 0.97) or low- (χ² = 1.20) altitude Bolivian subjects, suggesting that the high frequencies of Ala allele of Bolivian subjects had no association with BMI. The average BMI values for high- and low-altitude Bolivian Ala carriers are
24.1 ± 0.5 and 24.4 ± 0.6, respectively. They are almost the same as that (24.4 ± 0.5) of Japanese (Ala carriers, 8.3%). It has been reported that a relatively common Pro12Ala substitution in PPAR γ 2 is associated with lower BMI in Finns [6]. However, the Pro12Ala PPAR γ 2 variant associates with higher BMI in two independent Caucasian populations [15], and no difference in BMI was observed between subjects with Ala12 and those without it in Japanese populations [5]. Taken together, our results suggest that the direct relation of the PPAR γ 2 gene polymorphism to obesity may be little or not very strong in Bolivians, although the gene polymorphism in humans moderately reduces its transcriptional activity, and thus it is thought to be one of the thrifty genes.

Acknowledgment

We thank Ms. Makiko Senda and Ms. Rie Hirose for their helpful technical supports. We also thank Dr. Makoto Yoshioka for his excellent suggestions.

References

ポリビア高地および低地住民のperoxisome proliferator activated receptor γ2の遺伝子多型性

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要 旨： Peroxisome proliferator activated receptor γ (PPARγ)は、核内に存在する受容体で、転写因子でもあり、脂肪細胞の分化、脂肪細胞特異的遺伝子の発現、脂質代謝およびインスリン感受性を含む受容体などとして機能している。PPARγにはアイソフォームPPARγ1とPPARγ2が存在する。PPARγ1にはN末端から12番目のアミノ酸がProからAlaの変異型が存在しており、PPARγ2の転写因子としての機能に変化が生じると指摘されている。そして、肥満や糖尿病などの発症の頻度と関連性が議論されており、β3アドレナリン受容体遺伝子の仲間として候補遺伝子の一つにあげられている。本研究では南米アンデス地域の海拔4000m以上の高地に住むnativeポリビア人および300m以下の低地nativeポリビア人のPPARγ2のPro12Ala遺伝子多型性を測定し、長期にわたって異なる生活スタイルを取ってきた両ポリビア原住民の遺伝子型の比較と肥満との関連性の考察、また他民族の遺伝子型との比較を行った。その結果、ポリビア高地および低地住民の間でPPARγ2のPro12Alaの遺伝子型に統計的に大きな有意差は見られなかったが、肥満指数(BMI)と遺伝子型との関連性は高地、低地の住民と変わらなかった。したがって長期にわたる生活週間の差が、ポリビア高地、低地住民のこの遺伝子型の差に反映されたかもしれないが、PPARγ2が肥満関連遺伝子であるとの説は今回の研究結果からは否定的なものであった。他民族との比較では、ポリビア人のPro12Alaの変異頻度は高く特異的であった。

キーワード： PPARγ2，候補遺伝子，遺伝子多型性，肥満。