The Relationship Between Early-onset Childhood Obesity and *POMC* and *UCP3* Gene Variance

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Background. Human Uncoupling Protein 3 *(UCP3)* is a new candidate gene for human obesity. *UCP3* has been shown to be regulated by thyroid hormone, 3-adrenergic agonists, leptin, and fat feeding in rodents. Mutations in exon 3 (V102I) and exon 4 (R143X) were identified in obese and diabetic probands. Pro-opiomeleancortin *(POMC)*, another obese gene, plays a central role in α-MSH regulation of food intake by activating melanocortin-4-receptors in the brain. The *POMC* gene codes for the prohormone pro-opiomelanocortin, the hormone suspected of playing a role in appetite and body weight regulation.

Methods. We selected 100 obese and 300 non-obese children for analysis. Obesity was defined as a body mass index greater than or equal to the 95 percentile. To examine whether mutations in exon 3 (V102I) and 4 (R143X) of the uncoupling protein gene might be factors for obesity in Taiwanese children, the target DNA fragments were amplified by polymerase chain reaction, and the genotypes were defined by restriction fragment length polymorphism.

Results. No significant differences in the frequency of these mutations were found between obese and non-obese children.

Conclusions. The results indicate that those mutations of the *UCP3* and *POMC* genes do not play a role in the development of obesity in Taiwan's children. **(Mid Taiwan J Med 2001;6:216-22)**

Key words

obesity, polymorphism, *POMC, UCP3*

INTRODUCTION

Obesity is a steadily increasing health problem that causes complications such as type 2 diabetes, hypertension, atherosclerosis and neoplasms [1]. Obesity appears to originate in childhood [2,3], and reports indicate that the prevalence of obesity in childhood has been increasing in recent years in both Taiwan [4] and western counties [5,6].

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Data from studies on family and twins have indicated that genetic factors significantly contribute to the development of obesity [7,8]. About 40% to 70% of the variation in obesity-related phenotypes, such as body mass index, sum of skinfold thickness, and fat mass, is heritable [9,10]. The recent isolation and cloning of obese genes that induce obesity and diabetes in mice when mutated has attracted particular attention [11].

Uncoupling proteins (UCPs) play an important role in generating heat and burning calories. They are inner mitochondria membrane transporters that dissipate the

proton gradient. This uncoupling oxidative phosphorylation of ADP and ATP leads to the generation of heat [12]. Three distinct UCPs have been identified. *UCP1* is expressed exclusively in brown adipose tissue [13]. *UCP2* is present in several tissues, including the liver, skeletal muscle and white adipose tissue [12,14]. *UCP3* is expressed predominately in skeletal muscle [15,16]. Surprisingly, *UCP2* and *UCP3* gene expressions have been shown to be induced by fasting and are suppressed by feeding [15,17]. These two genes have been localized within 150kb of each other on chromosome 11q13 which consists of six coding exons [18,19]. *UCP3* is a new candidate gene for human obesity and has been found to have genetic linkage to the *UCP2/UCP3* locus [20,21]. It is regulated by thyroid hormone, β3-adrenergic agonists, leptin and fat feeding in rodents [22,23]. To date, mutations in *UCP3* exon 3 (G304A) and exon 4 (C427T) were identified in obese and diabetic probands [24], and the reported genetic defect directly associated with human obesity has been congenital leptin deficiency [11,25]. It has been shown that the G304A polymorphism in exon3 results in a conservative amino acid substitution of a valine by an isoleucine at residue 102 (V102I), and that the C427T polymorphism results in a premature stop codon at residue 143 (R143X) in the third, matrix-orientated, loop [24].

Another obese gene is pro-opiomeleancortin *(POMC)*. In antagonist models, it plays a central role in α-MSH regulation of food intake by activation of the brain melanocortin-4-receptor *(MC4R)* [26].

Comuzzie identified a major quantitative trait locus determining serum leptin levels and fat mass located on 2p21, in close proximity to the POMC loucs. These findings led to the proposal of an association between *POMC* and human obesity [27]. The endogenous ligand for *MC4R* is α-MSH, a peptide cleaved from *POMC*. Sequential cleavage of this precursor also generates melanocortin peptides β-MSH, γ-MSH, adrenocorticoid hormone (ACTH) and β-endorphin [28]. Expression of the *POMC* gene in the hypothalamus and brainstem produces melanocortigenic neurotransmitters, and expression in the pituitary gland produces melanocortin hormones [29]. The association between *POMC* and human obesity has been proposed by Yaswen [30] who generated *POMC* knockout mice lacking all *POMC*-derived peptides. These mice became obese with a time course and severity comparable to the *MC4R* knockout mice [20]. A recent study has reported that severe early onset obesity, adrenal insufficiency, and red hair pigmentation is caused by *POMC* mutation in humans [31]. The researchers found two mutations in exon 3 and one mutation in exon 2. In exon 2, the C3804A mutation abolished *POMC* translation. In exon 3, a G→T polymorphism at nucleotide 7013 resulted in a premature termination at codon 79. Truncation of the *POMC* protein at codon 79 predicted complete absence of ACTH, β-MSH, and α-endorphin, which are encoded further downstream [31].

In this study, we screened two mutations in exon 3 and exon 4 of the *UCP3* gene, and two mutations in exon 2 and exon 3 of the *POMC* gene by polymerase chain reaction (PCR), and restriction fragment length polymorphism (RFLP) analysis. We wanted to evaluate the frequency of polymorphic positions in the *UCP3* and *POMC* genes and to analyze those variations and their relationship to early-onset obesity in Taiwan's school children.

MATERIALS AND METHODS

Study Design and Population

A cross-sectional study of 2, 230 first graders was conducted from March to April, 2000 in Taichung city, Taiwan. We further selected 100 obese and 300 non-obese children based on their body mass index (BMI). The measure of fatness was determined by BMI which was calculated as follows; BMI = weight (kg)/height (m^2). Obese children were defined as those with a BMI greater than or equal to the 95 percentile, and the BMI of non-obese

children as beeing between the 45th and 55th the percentiles [32].

All selected students were given a structured questionnaire with an invitation letter briefly describing the study and instructions for filling out the questionnaire. Those who agreed to participate in the study would sign their consent before they filled out questionnaires. Information collected consisted of demographic data and medical history.

The clinical examination included measurement of sitting blood pressure, height and weight. The students fasted for more than eight hours on the day of examination. Blood samples were sent to the laboratory at the China Medical College Hospital for analysis.

Analysis of *UCP3* and *POMC* Genes

Genomic DNA was isolated from 3 mL of peripheral blood as previously described [33]. The following primers were used to amplify genomic DNA and to generate products containing the V102I mutation of exon 3 and the R143X mutation of exon 4: *UCP3*-E3: 5'-TCT TGC CTT CCC ATC TGA GT-3'; 5'-GGG ACC TGG TCA CTC ACT GTC CGC GCC TTT GGG GGT GTC CAC C-3'; and *UCP3*-E4: 5'-CGT CCA TAG TCC CGC TGT AT-3'; 5'-AGC CTC ACT ACC CGG ATT TT-3'. The reaction mixture (50 μL) contained 100 ng to 500 ng genomic DNA, 1 μM of each primer, 200 μM dNTPs, 2.5 mM $MgCl_2$ and 1U Taq DNA polymerase *(ProTech)*. The PCR reactions were denatured for 5 min at 95 °C and performed for 35 cycles. To amplify *UCP3* exon 3, each cycle consisted of three segments: denaturing at 95 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 2 min, with an additional extension period of 5 min at 72 °C. To amplify exon 4 of the *POMC* gene, the annealing temperature was 50 °C.

To determine the V102I mutation, the PCR products were digested with *Xcm*I (*BioLabs* Inc.), a 10 μL reaction mixture containing 1 μg of DNA and 1 unit of restriction enzyme incubated for 12 hours at 37 °C. The R143X mutation was determined by *Bsl* I (*BioLabs* Inc.) digestion and incubated for 12 hours at 55 °C. Digested PCR products were loaded onto a 3.5% agarose gel and visualized with ethidium bromide under UV light.

To analyze the *POMC* gene, the following primers were used to amplify genomic DNA and generate PCR products containing exon 2 (C→A) and exon 3 (G→T). *POMC*-E2: 5'-GCT CAA GGT CCT TCC TGG TG-3'; 5'-GCC CTG GAT TGA ATC ACG CC-3'; and *POMC*-E3: 5'-CGG CCA GGG CCT AGG CGC AG-3'; 5'-TCG TCC TCG GCG CCG TTA GG-3'. The reaction mixture (50 μL) contained 100 ng to 500 ng genomic DNA, 1 μM of each primer, 200 μM dNTPs, 2.5 mM $MgCl_2$ and 1U Taq DNA polymerase (*ProTech*).

PCR amplification of the *POMC* gene was similar to *UCP3* , except in exon 2, for which the annealing temperature was 52 °C, and in exon 3, where the annealing temperature was 56 °C. To detect the mutation of exon 2, the PCR products were digested with *Sph*I (*BioLabs* Inc.); and for exon 3, the PCR products were digested with *Bfa*I (*BioLabs* Inc.). The reaction mixture contained 1 μg of DNA and 1 unit of restriction enzyme incubated for 12 hours at 37 °C. The digested PCR products were then analyzed on a 3% agarose gel and visualized with ethidium bromide.

RESULTS

The *UCP3* product was amplified from genomic DNA from 100 obese and 300 non-obese children. The PCR products of exon 3 were 273 bp (Fig. 1 lane 1). After digestion with *Xcm*I, the products of the G/G wild-type were 116 bp and 157 bp fragments (Fig. 1 lane 3-4). The A/A mutant-type products (G304A) were 116 bp, 110 bp and 47 bp fragments. The PCR product of *UCP3* exon 4 was a 152 bp fragment (Fig. 2, lane 1). Following *Bsl*I digestion, three bands of 18 bp, 62 bp and 72 bp for the C/C wild-type (Fig. 2, lanes 2-4), and two bands of 18 bp and 134 bp for the T/T mutant-type (C427T) were seen.

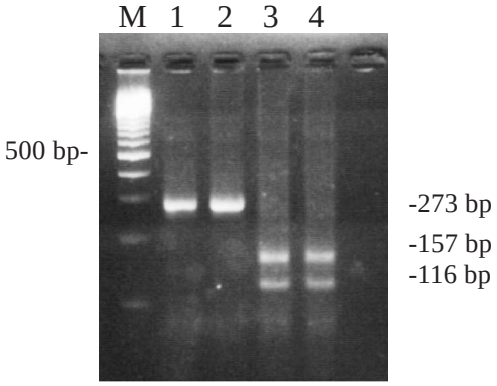

Fig. 1 PCR analysis of *UCP3* exon 3 mutation. The mutation region was amplified by PCR, which resulted in a 273 bp fragment (lane 1). After digesting with *Xcm*I, the wild-type was cleaved into 116 bp and 157 bp fragments (lanes 3 & 4) M, marker, 100 bp ladder.

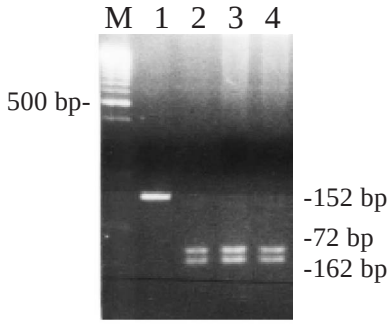

Fig. 2 PCR analysis of *UCP3* exon 4 mutation. The mutation region was amplified by PCR, which resulted in a 152 bp fragment (lane 1). After digesting with *Bsl*I, the wild-type was cleaved into 18 bp, 62 bp and 72 bp fragments (lanes 2 & 4) M, marker, 100bp ladder.

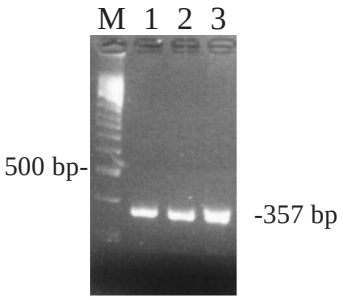

Fig 3 PCR analysis of *POMC* exon 2 mutation. The mutation region was amplified by PCR, which resulted in a 357 bp fragment (lane 1). After digesting with *Sph* I, the wild-type remained uncut (lanes 2 & 3). M, marker, 100 bp ladder.

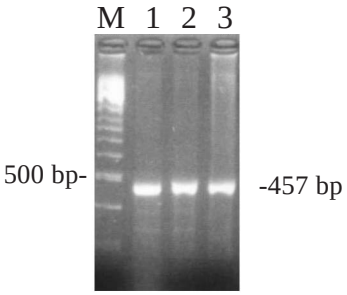

Fig 4 PCR analysis of *POMC* exon 3 mutation. The mutation region was amplified by PCR, which resulted in a 457 bp fragment (lane 1). After digesting with *Bfa*I, the wild-type was cleaved into 11 bp and 446 bp fragments (lane 2-3). M, marker, 100 bp ladder.

For analysis of the *POMC* gene, a 357 bp (Fig. 3, lane 1) fragment was amplified from *POMC* exon 2. After digesting the products with *Sph*I, the C/C wild type showed one band of 357 bp (Fig. 3, lanes 2 & 3). The A/A mutant type (C3804A) revealled two bands of 266 bp and 91 bp. The PCR product of *POMC* exon 3 was 457 bp (Fig. 4, lane 1). After digesting with *Bfa*I, the G/G wild-type product showed 446 bp and 11 bp fragments (Fig. 4, lanes 2 & 3). The product of the T/T mutant type (G7013T) showed 258 bp, 87 bp and 11 bp fragments. In addition, the PCR products from *UCP3* and *POMC* were sequenced in order to confirm the restriction fragment length polymorphism results (data not shown).

Afteve all, we didn't find mutations in either exon 2 or exon 3 of the *POMC* gene, nor exon 3 or exon 4 of the *UCP3* gene.

DISCUSSION

Some previous studies have found a link between body weight or energy expenditure and the *UCP-2/UCP-3* locus [19,24,34,35]. For example, the A/A homozygous frequency in the V102I mutation of exon 3 is 4%, and the G/A heterozygous frequency is 28% in African Americans [24]. In the Mende tribe of Sierra Leone, the A/A homozygous frequency is 3%, and the G/A heterozygous frequency is 21% [24]. But in our study, the V102I mutation was not found in Caucasian Americans [25]. The R143X mutation of exon 4 was not

detected in our population, which is similar to findings in African Americans and Mende tribes men [24].

Recently, three different mutations in the *POMC* gene were identified in two unrelated children that lead to early-onset extreme obesity, adrenal insufficiency, and red hair pigmentation [31]. Some studies identified novel polymorphisms and mutations in the *POMC* gene, yet none were readily associated with the phenotype [36] nor related to obesity [37].

In the present study, we examined whether genetic variation in *UCP3* and *POMC* is associated with obesity in Taiwan's school children. We screened for two of the previously identified mutations in *UCP3* and *POMC*, however, we didn't find them in our population. This could be due to differences in ethnic backgrounds in these populations. Differences in age and diet may partially explain the causes of these disparate results. Other environmental or genetic cofactors might also be required to influence the development of obesity.

There was no significant finding in the mutation frequency in *UCP3* and *POMC*. This might indicate that those mutation sites are not related to obese children in Taiwan. Further evaluation of other mutations or polymorphic sites related to obesity may be necessary to determine the genetic link between obesity and Taiwan's children.

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*POMC*及*UCP3*基因多形性與台灣學童肥胖相關性之研究

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背景　　爲瞭解台灣學童之肥胖情形與*POMC*及*UCP3*之相關性。

方法　　本研究以台中市之國小學童爲樣本，收集其血液樣本粹取DNA，以BMI指數爲指標選取肥胖者100名及非肥胖者300名爲研究對象，利用聚合酵素鏈鎖反應(PCR)及限制酵素片斷多形性(RFLP)等方法來判定基因之基因型。

結果　　肥胖者在*POMC*及*UCP3*兩基因之基因多形性(polymorphism)的分佈上與非肥胖者並無差異。

結論　　*POMC*及*UCP3*兩基因在本研究所分析之基因多形性與台灣學童的肥胖無顯著的相關性。（中台灣醫誌 **2001;6:216-22**）

關鍵詞

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