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Integrative Study on PPARGC1A: Hypothalamic Expression of Ppargc1a in *ob*/*ob* Mice and Association between PPARGC1A and Obesity in Korean Population

Mee Suk Hong[1,*], Hye Kyung Kim[2,*], Dong Hoon Shin[3], Dae Kyu Song[3], Ju Yeon Ban[1], Bum Shik Kim[1,4] & Joo-Ho Chung[1]

[1]Kohwang Medical Research Institute, School of Medicine, Kyung Hee University, Seoul 130-701, Korea
[2]Department of Food and Biotechnology, Hanseo University, Seosan 356-706, Korea
[3]Department of Preventive Medicine, Physiology, School of Medicine and Institute for Medical Genome Research, Keimyung University, Daegu 700-712, Korea
[4]Department of Thoracic and Cardiovascular Surgery, School of Medicine, Kyung Hee University, Seoul 130-701, Korea
Correspondence and requests for materials should be addressed to J. H. Chung (jhchung@khu.ac.kr; joohochung@empal.com)
*Mee Suk Hong and Hye Kyung Kim contributed equally ro this work.

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Abstract

Obesity is an increasing worldwide health problem that is strongly related to the imbalance of food intake and energy metabolism. It was well-known that several substances in the hypothalamus regulate food intake and energy metabolism. We planned an integrative study to elucidate the mechanism of the development of obesity. Firstly, to find candidate genes with the marvelous effect, the different expression in the hypothalamus between *ob*/*ob* and 48-h fasting mice was investigated by using DNA microarray technology. As a result, we found 3 genes [*peroxisome proliferator activated receptor*, *gamma*, *coactivator 1 alpha* (*Ppargc1a*), *calmodulin 1* (*Calm1*), and *complexin 2* (*Cplx2*)] showing the different hypothalamic expression between *ob*/*ob* and 48-h fasting mice. Secondly, a genetic approach on *PPARGC1A* gene was performed, because PPARGC1A acts as a transcriptional coactivator and a metabolic regulator. Two hundred forty three obese female patients with body mass index (BMI) ≥ 25 and 285 control female subjects with BMI 18 to < 23 were recruited according to the Classification of Korean Society for the Study of Obesity. Among the coding single nucleotide polymorphisms (cSNPs) of *PPARGC1A*, 2 missense SNPs (rs8192678, Gly482Ser; rs3736265, Thr612Met) and 1 synonymous SNP (rs3755863, Thr528Thr) were selected, and analyzed by PCR-RFLP and pyrosequencing. For the analysis of genetic data, chi-square (X^2) test and EH program were used. The rs8192678 was significantly associated with obese women ($P < 0.0006$; odds ratio, 1.5327; 95% confidence interval, 1.2006-1.9568). Haplotypes also showed significant association with obese women ($X^2 = 33.28$, $P < 0.0008$). These results suggest that *PPARGC1A* might be related to the development of obesity.

Keywords: Hypothalamus, Microarray, *ob*/*ob* Mice, Obesity, Polymorphism, PPARGC1A

Development of obesity is related to modern lifestyles, such as increased food intake and reduced physical activity. Imbalance of energy homeostasis can lead to obesity, anorexia, and other diseases[1]. Energy homeostasis is influenced by physiological events that occur in both peripheral tissues and the central nervous system (CNS)[1,2]. Several researchers have been reported the relationship between the hypothalamus and the regulation of energy homeostasis or appetite[2-4]. Leptin as a hypothalamic neurodomulator regulates feeding behavior and energy metabolism[4,5].

To date, the microarray technology has been used for the finding of candidate susceptibility genes in several diseases. Delparigi *et al*.[6] reported the differential gene expression in the hypothalamus of obese and lean humans. Soukas *et al*.[7] showed the gene expression profiles in white adipose tissue of *ob*/*ob* mice. Nadler *et al*.[8] identified the difference expression in adipose tissue from lean, obese, and obese-diabetic mice. Thus, the microarray technology develops recent emerging fields such as nutrigenomics[9-11].

In this study, we designed an integrative study to elucidate the mechanism of the development of obesity. To identify candidate gene expression profiles in

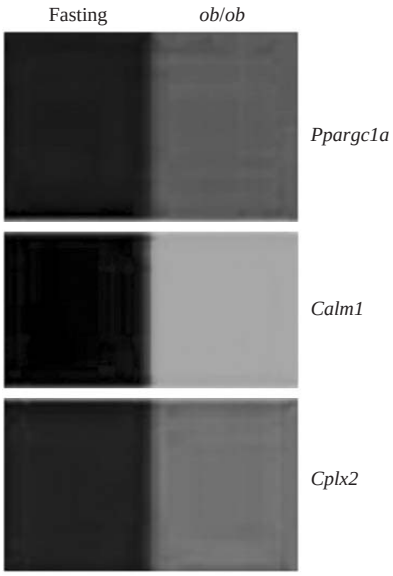

Figure 1. Clustering of selected genes. Fasting, the expression of the hypothalamus of 48-h fasting mice; *ob*/*ob*, the expression of the hypothalamus of *ob*/*ob* mice. *Ppargc1a, peroxisome proliferator activated receptor, gamma, coactivator 1 alpha*; *Calm1, calmodulin 1*; *Cplx2, complexin 2*. Two independent experiments were performed.

the hypothalamus of *ob*/*ob* mice, DNA microarray technology was used. Next, a case-control study for the candidate gene was performed. Two hundred forty three obese and 285 control female subjects were recruited and analyzed.

Analysis and Clustering of Microarray Profiles

The *ob*/*ob* mice revealed 11 up- and 30 down-regulated genes (>2 fold), compared with the control mice. In the 48-h fasting mice, 5 genes were up-regulated and 5 genes down-regulated. These genes are related to the immune response, lipid metabolism, and signal transduction (data not shown). When the hypothalamic expression profile of *ob*/*ob* mice was compared to that of 48-h fasting mice, we found a few distinguished expression genes between two groups, such as *peroxisome proliferator activated receptor, gamma, coactivator 1 alpha* (*Ppargc1a*), *calmodulin 1* (*Calm1*), and *complexin 2* (*Cplx2*) (Figure 1).

Genetic Analysis of *PPARGC1A* Polymorphism in Obesity

Next, we focused the *PPARGC1A* gene for a genetic study, because the protein encoded by this gene is involved in energy metabolism and development of obesity. Therefore, we investigated the association between three coding single nucleotide polymorphisms (cSNPs) of *PPARGC1A* and obesity in Korean population. Three SNPs [rs8192678 (Gly482Ser), rs3736265 (Thr612Met), and rs3755863 (Thr528Thr)] were genotyped in 258 control (BMI 18 to <23) and 243 obese (BMI≥ 25) female subjects. The genotype and allele frequencies, odds ratio (OR), 95% confidence interval (CI) and *P* value are shown in Table 1. Genotype distributions of three SNPs were all in Hardy-Weinberg equilibrium ($P>0.05$). The rs8192678 was associated with obese women (OR=1.5327, 95 % CI=1.2006-1.9568). Frequencies of AA genotype and A allele of the rs8192678 in the obesity group were about 10% higher than those in the control group, respectively (Table 1). The results suggest that A allele is a risk factor of the development of obesity in female Korean population. The rs3736265 and rs3755863 were not significantly associated with obesity (Table 1).

We calculated the sample power of the significant SNP (rs8192678) using G*Power computer software. We had 0.889, assuming an α-level of 0.05. Thus, the significant SNP in our case-control study was sufficiently powerful for determining a positive association.

Haplotype Analysis

Haplotype analysis was performed on three SNPs of *PPARGC1A*. Among eight haplotypes, the frequency of most common haplotype (GGC) in control and obesity subjects was 44.7% and 39.5%, respectively. The frequency of second common haplotype (AAC) in control and obesity subjects was 25.8% and 30.2%, respectively. The AAT frequency in the obesity group (16.9%) was higher that in the control group (12.6%). The frequency of AGC haplotype in the obesity group (3.7%) was about three times higher than that in the control group (1.3%) (Table 2). Statistical analysis showed that significant association in haplotype frequency is present between control and obesity subjects ($\chi^2=33.28$, $P<0.0008$) (Table 2).

Discussion

Through clustering data from the hypothalamic expression profiles of *ob*/*ob* and 48-h fasting mice, we identified 3 distinguished expression genes (*Ppargc1a*, *Calm1*, and *Cplx2*). Among 3 differential expression genes, we focused on the *PPARGC1A* gene. PPARGC1A protein (Q9UBK2) consists of 798 amino acids and molecular mass of PPARGC1A is 91,027 Da. Amino acids from 677 to 753 comprise RRM domain, 293 to 339 PPARG interaction region, and 144 to 148 LXXLL motif (UniProt, http://beta.uniprot.

Table 1. Genotype and allele frequencies of polymorphisms of *PPARGC1A* gene.

		rs8192678 (%)			rs3755863 (%)			rs3736265 (%)		
		GG	GA	AA	GG	GA	AA	CC	CT	TT
Genotype distribution	Control (n=258)	102 (35.79)	135 (47.37)	48 (16.84)	75 (26.41)	118 (41.55)	91 (32.04)	191 (67.02)	83 (29.12)	11 (3.86)
	Obesity (n=243)	60 (24.69)	117 (48.15)	66 (27.16)	48 (19.75)	116 (47.74)	79 (32.51)	162 (66.67)	72 (29.63)	9 (3.70)
	[a]*P* value	*P*=0.0028			*P*=0.1634			*P*=0.9889		

		rs8192678 (%)		rs3755863 (%)		rs3736265 (%)	
		G	A	G	A	C	T
Allele frequency	Control (n=258)	337 (59.33)	231 (40.67)	268 (47.18)	300 (52.82)	465 (81.58)	105 (18.42)
	Obesity (n=243)	237 (48.77)	249 (51.23)	212 (43.62)	274 (56.38)	396 (81.48)	90 (18.52)
	[b]*P* value	*P*=0.0006		*P*=0.2471		*P*=0.9675	
	OR (95%CI)	1.5327 (1.2006-1.9568)		1.1546 (0.9051-1.4729)		1.0065 (0.7369-1.3748)	

Numbers in parentheses indicate percentages. *PPARGC1A, peroxisome proliferator activated receptor, gamma, coactivator 1 alpha*. n, number; OR, odds ratio; CI, confidence interval. Statistical analysis was performed by the chi-square test. [a]Control vs. Obesity using the chi-square test with 2 × 3 contingency table. [b]Control vs. Obesity using the chi-square test with 2 × 2 contingency table.

Table 2. Haplotype frequencies of three SNPs (rs8192678 G/A, rs3755863 G/A, and rs3736265 C/T) of *PPARGC1A* gene.

Haplotype	Frequency	
	Control	Obesity
GGC	0.447	0.395
GAC	0.096	0.079
AGC	0.013	0.037
AAC	0.258	0.302
GGT	0.005	0.000
GAT	0.046	0.012
AGT	0.006	0.004
AAT	0.126	0.169
	*P=0.0008, d.f.=7, χ^2=33.28	

PPARGC1A, peroxisome proliferator activated receptor, gamma, coactivator 1 alpha. d.f., degree of freedom. **P* value for overall difference in haplotype distribution between control and obesity was calculated by EH program.

org; SwissProt, http://www.expasy.org). PPARGC1A is a transcriptional coactivator and a key regulator of energy metabolism, and activated by cellular signals controlling energy and nutrient homeostasis[12-14]. Several researchers reported that dysfunction of PPARGC1A may be related to the pathophysiology of cardiac diseases, diabetes or insulin resistance[15-17]. Vimaleswaran *et al.*[18] reported that the Thr394Thr polymorphism of *PPARGC1A* was associated with type 2 diabetes in Asian Indians. Vimaleswaran *et al.*[19] also reported the Thr394Thr polymorphism associated with increased body fat. However, Nelson *et al.*[20] showed that rs8192678, rs3755863, and rs3736265 polymorphisms of *PPARGC1A* were not associated with type 2 diabetes or BMI among Hispanic and non Hispanic Whites from Colorado. In this study, we investigated three SNPs of *PPARGC1A* (rs8192678, Gly482Ser; rs3755863, Thr528Thr; rs3736265, Thr612Met). The rs8192678 is located on exon 8, and is a missense SNP (rs8192678) with 0.411 heterozygosity. The A and G allele frequencies are reported to be 0.367 and 0.633 in European, 0.455 and 0.545 in Chinese, 0.455 and 0.545 in Japanese, 0.017 and 0.983 in Sub-Saharan African, and 0.211 and 0.789 in African American, respectively (http://www.ncbi.nlm.nih.gov/SNP). The A and G allele frequencies in female Korean population were 0.407 and 0.593, which are similar to those in Asians. The rs3755863 is located on exon 8, and is a synonymous SNP (rs3755863) with 0.465 heterozygosity. The A and G allele frequencies are reported to be 0.433 and 0.567 in European, 0.444 and 0.556 in Chinese, 0.455 and 0.545 in Japanese, 0.200 and 0.800 in Sub-Saharan African, and 0.333 and 0.7667 in African American, respectively (http://www.ncbi.nlm.nih.gov/SNP). The A and G allele frequencies in female Korean population were 0.528 and 0.472. The rs3736265 is located on exon 9, and is a missense SNP (rs3736265) with 0.201 heterozygosity.

The T and C allele frequencies are reported to be 0.083 and 0.917 in European, 0.167 and 0.833 in Chinese, 0.148 and 0.852 in Japanese, 0.092 and 0.908 in Sub-Saharan African, and 0.092 and 0.908 in African American, respectively (http://www.ncbi.nlm.nih.gov/SNP). The T and C allele frequencies in female Korean population were 0.184 and 0.816, which are also similar to those in Asians. Our results revealed that the rs8192678 SNP was associated with obesity in female Korean population, whereas the rs3755863 and rs3736265 SNPs were not (Table 1). Pihlajamäki *et al*.[21] reported that the haplotypes of *PPARGC1A* were associated with glucose tolerance, BMI, and insulin sensitivity in offspring of patients with type 2 diabetes. Our study also showed that the haplotype frequencies of *PPARGC1A* was associated with obesity in female Korean population ($\chi^2=33.28$, $P<0.0008$) (Table 2).

In conclusion, we found the obesity-related genes by using animal models and microarray technology. Among the genes, the *PPARGC1A* gene was significantly associated with obesity in female Korean population. These results suggest that *PPARGC1A* might be related to the development of obesity.

Materials & Methods

Animals and DNA Microarray

Genetically obese (*ob*/*ob*), 48-h fasting, and wild-type mice were housed at standard temperature (22 ± 3°C) and under a 12-h light/dark cycle (lights on at 07 : 00 h). The *ob*/*ob* and control mice were with free access to food and water, while the 48-h fasting mice were restricted with food. Mice were killed, and then the hypothalamus was isolated and homogenized. The total RNA was extracted using Mini RNA isolation II™ kit from Zymo Research (CA, USA). All procedures were performed in accordance with the international guidelines of Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1995) and Animal Care Committee of Medical College, Kyung Hee University.

Scanning and Clustering

The 3DNA™ Array 350 kit (Genisphere, PA, USA) was used for 7.4 K TwinChip™ microarray (Digital Genomics, Seoul, Korea). In brief, the hybridized microarray was scanned with a confocal laser scanning microscope (ScanArray 5000; Packard Inc., CT, USA) at 532 nm for Cy3 and 635 nm for Cy5. Using ArrayAssist Software Version 2.0 (Stratagene, TX, USA), gene expression data were filtered and normalized. Cluster images were created in TreeView[22].

Study Subjects

Two hundred forty three obese female patients with BMI ≥ 25 [36.4 ± 9.6 (mean ± SD) years] and 285 control female subjects with BMI 18 to < 23 (31.2 ± 7.3) were recruited according to the Classification of Korean Society for the Study of Obesity. Body weight and height of each subject were measured. BMI was defined as weight in kilograms divided by the square of height in meters (kg/m^2). To eliminate ambiguous effect, subjects with underweight (BMI < 18) and moderately obese (BMI 23 to < 25) were excluded in this study. Patients with hypertension, diabetes, hyperlipidemia, stoke, and cardiac diseases were also excluded. All studies were carried out according to the Declaration of Helsinki guidelines. Written informed consent was obtained from each subject. This study was approved by the Ethics Review Committee of the Medical Research Institute, School of Medicine, Kyung Hee University.

Genotyping

Genomic DNA was extracted from DNA isolation kit for mammalian blood (Macherey-Nagel GmbH & Co., Düren, Germany). For an association study, we selected 3 SNPs (rs8192678, rs3755863, and rs3736265) within the *PPARGC1A* gene region using the following websites: (1) human SNP websites (http://www.ensembl.org, www.ncbi.nlm.nih.gov/SNP) (2) HapMap database (http://www.hapmap.org) (3) tag SNPs site (http:// broad.mit.edu/mpg/tagger). Two missense SNPs (rs8192678 and rs3736265) was selected. Among missense SNPs, the SNPs with unknown or low heterozygosity (below 0.03) were excluded. One SNP (rs3755863) was chosen with highest heterozygosity among synonymous SNPs (www.ncbi.nlm.nih.gov/SNP). The rs8192678 and rs3755863 SNPs were analyzed by PCR-RFLP with restriction enzymes (*Msp*I and *Cac*8I), respectively. The primers for each SNP were made: for rs8192678 (329 bp), 5′-ttggtggtg acacagaatc-3′ (forward), 5′-ccttgcagcacaagaaaaca-3′ (reverse); for rs3755863 (361 bp), 5′-ttgggtggtgacaca gaatc-3′ (forward), 5′-agagactttggaggcaagca-3′ (reverse). The 329 bp PCR products of rs8192678 were cleaved by *Msp*I, resulting in two fragments (220 and 109 bp) for homozygote G/G, three fragments (329, 220, and 109 bp) for heterozygote G/A, and a single band (329 bp) for homozygote A/A. The 361 bp PCR products of rs3755863 were cleaved by *Cac*8I, resulting in four fragments (258, 82, 16, and 5 bp) for homozygote G/G, five fragments (340, 258, 82, 16, and 5 bp) for heterozygote G/A, and three fragments (340, 16, and 5 bp) for homozygote A/A. The other SNP (rs3736265) was analyzed by pyrosequencing (BMS, Seoul, Korea). The specific primers were made: for

rs3736265 (150 bp), 5′-aaatgacatgcctcattacc-3′ (forward), 5′-gtctcctcttttcgtttttc-biotin-3′ (reverse), and 5′-ggagaatttcggtgc-3′ (sequencing primer).

Statistical Analysis

All SNPs were tested Hardy-Weinberg expectations before further analysis (http://bioinfo.iconcologia.net/index.php). Differences in the genotype and allele frequencies were examined using the chi-square (χ^2) test. The OR and 95% CI were also calculated. The EH program was used for the analysis of haplotype[23]. Statistically difference was considered at the 5% level of significance. Power analysis was performed using G*Power computer software[24]. The SAS Statistical Software Package (release 8.02; SAS Institute Inc, NC, USA) was used.

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