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# Genetic Variations of Eight Candidate Genes in Korean Obese Group

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*ABSTRACT* : **Obesity is a complex metabolic disorder with a strong genetic component. There are many candidate genes for obesity and its related phenotypes. We studied genetic variations between Korean obese and lean groups. Polymorphisms investigated were the *Msp* I polymorphism of the $\alpha_{2A}$-adrenergic receptor ($\alpha_{2A}$-AR) gene, the *Mnl* I polymorphism of the $\alpha_2$-adrenergic receptor ($\alpha_2$-AR) gene, the *Bst*O I polymorphism of the $\beta_3$-adrenergic receptor ($\beta_3$-AR) gene, the *Pml* I polymorphism of the lamin A/C (LMNA) gene, the *Hga* I polymorphism of the clearance receptor (NPRC) gene, the the *Msp* I polymorphism of the leptin gene, *Bcl*I polymorphism of the uncoupling protein 1 (UCP1) gene and the *Hha* I polymorphism of the fatty acid binding protein 2 (FABP2) gene. Among these genetic markers, *Pml* I polymorphism at the LMNA gene and *Bcl* I polymorphism at the UCP1 gene were significantly associated with obesity. However, further studies are required whether thease findings are reproduced in large population, although two polymorphisms might be useful as genetic markers in the ethiology of obesity in Korean population.**

**Keywords: candidate genes, obesity and polymorphism**

## Introduction

Obesity is a highly prevalent disorder that is associated with decreased longevity and increased morbidity from a variety of disoders and diseases including hyperglycemia, hyperlipidemia, hypertension, and cardiovascular disease (Kissebah *et al.,* 1989). The identification of genes involved in human obesity has been unsuccessful so far, probably because of difficulties in judging which factors are due to shared genes and which to shared environment or both. There are many candidate genes for obesity and its related phenotypes. Some genes are candidates for obesity because mutations in them cause rare genetic syndromes affecting adipocyte differentiation (Bouchard *et al.,* 1998; Robert *et al.,* 2000). The recognition of familial obesity led to the notion that even in sporadic cases, genetic factors might contribute to the disease susceptibility. In the case of complex diseases that do not exhibit a clear pattern of familial aggregation, the candidate gene approach, which tests the role of known genes selected for their potential implication in the pathophysiological process, is a widely used strategy (Cambien *et al.,* 1997; Lander *et al.,* 1996). This study is a large case-control study for Korean subjects. It designed to identify genetic factors involved in the predisposition to obesity. In this study, the genetic factors involved in the predisposition to obesity were examined among Korean population. The results of an investigation using eight candidate genes: the $\alpha_{2A}$-adrenergic receptor ($\beta_{2A}$-AR), the $\beta_2$-adrenergic receptor ($\beta_2$-AR), the $\beta_3$-adrenergic receptor ($\beta_3$-AR), the lamin A/C (LMNA), the clearance receptor (NPRC), the uncoupling protein 1 (UCP1), the leptin gene, and the fatty acid binding protein 2 (FABP2) genes were reported in this study.

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## Materials and methods

### Study subjects

One hundred and seventeen subjects were recruited from outpatients of Seoul Hygiene Hospital, Seoul,

Korea. The obese group consisted of 37 subjects with higher body mass index (BMI) than 26 kg/m$^2$, whereas the lean group consisted of 80 individuals with lower body mass index (BMI) than 26 kg/m$^2$ (Herzog *et al.*, 1993; Rutherford *et al.*, 1997; Zee *et al.*, 1995; Zee *et al.*, 1997). There were no significant differences in age and sex distributions between two groups.

### Genotyping

Genomic DNA was prepared from buffy coats of 5 ml blood after lysis of red blood cell (Sambrook *et al.*, 1989). The polymorphisms investigated in this study were a nucleotide substitution C-1291G in the $\alpha_{2A}$-adrenergic receptor ($\alpha_{2A}$-AR); a nucleotide substitution T164I in the $\beta_2$-adrenergic receptor ($\beta_2$-AR); a nucleotide substitution T64A in the $\beta_3$-adrenergic receptor ($\beta_3$-AR); a nucleotide substitution T1908C in the lamin A/C (LMNA); a nucleotide substitution A-55C in the clearance receptor (NPRC); a nucleotide substitution A-3826G in the uncoupling protein 1 (UCP1); a nucleotide substitution C315T in the leptin gene; and a nucleotide substitution A54T in the fatty acid binding protein 2 (FABP2) gene. PCR reactions were performed in a final volume of 50 ul (100 ng of genomic DNA, 20 pmol of each primers, 200 *u*M each of the four dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl, pH 8.4 and 2.5 unit of *Taq* DNA polymerase (Promega, Co. Ltd., Madison, USA). PCR reaction primer sequences and references were shown in Table 1. In the case of â$_2$-AR, the sequence of the forward primer 5'-GCTACTTTGCCATTACTTCAC CTT-3' and the reverse primer 5'-GTAGAAGGACACG ATGGAAGAGG-3' were designed from the full-length sequence of the $\beta_2$-AR gene (Genebank # AF203386) using Primer3 program (the Whitehead Institute for Biomedical Research/MIT center for genome research, U.S.A). Amplified PCR products were digested by each restriction enzymes, and visualized by agarose gel with ethidium bromide staining.

### Statistical analysis

Data were analyzed using the SAS version 6.12 statistical software (SAS Institute, Cary, North Carolina, USA). Allele frequencies were estimated by gene counting. Hardy-Weinberg equilibrium was tested by chi-square analysis. Genotype and allele frequencies were compared between Korean obese and lean groups by chi-square analysis. Genotypic odds ratios (OR) for disease, assuming a dominant or a recessive model, were computed by logistic regression analysis. Dominant model was defined by the comparison between MM genotype and (Mm + mm) genotypes (M, normal allele; m, disease allele). Recessive model was defined by the comparison between (MM + Mm) genotypes and mm genotype. A P value<0.05 was considered statistically significant.

## Results and discussion

The candidate genes examined in the present study was selected based on pathophysiological considerations and/or results previously reported in published data.

**Table 1.** Polymorphic sites and primer sequence of each candidate gene

| Genes | Polymorphic sites | Primer sequences | Reference |
|---|---|---|---|
| $\alpha_{2A}$-adrenergic receptor | *Msp* I RFLP | 5'-TCACACCGGAGGTTACTTCCCTCG-3'<br>5'-TCCGACGACAGCGCGAGTT-3' | Sergio *et al.*, 1997 |
| $\beta_2$-adrenergic receptor | *Mnl* I RFLP | 5'-GCTACTTTGCCATTACTTCACCTT-3'<br>5'-GTAGAAGGACACGATGGAAGAGG-3' | Present study |
| $\beta_3$-adrenergic receptor | *Bst*O I RFLP | 5'-CGCCCAATACCGCCAACAC-3'<br>5'-CCACCAGGAGTCCCATCACC-3' | Kristi *et al.*, 1997 |
| Lamin A/C | *Pml* I RFLP | 5'-GCAAGATACACCCAAGAGCC-3'<br>5'-ACACCTGGGTTCCCTGTTC-3' | Robert *et al.*, 2000 |
| Clearance receptor | *Hga* I RFLP | 5'-CACCGTCAATTACAAACACTTGGACAAGTCTAAC-3'<br>5'-CACCTTCCTCTTTCCTCCCCACTCTTCTCTCCA-3' | Sarzani *et al.*, 1999 |
| Uncoupling protein 1 | *Bcl* I RFLP | 5'-CCAGTGGTGGCTAATGAGAGAA-3'<br>5'-GCACAAAGAAGAAGCAGAGAGG-3' | Valve *et al.*, 1998 |
| Leptin | *Msp* I RFLP | 5'-CAGTCAGTCTCCTCCAAACA-3'<br>5'-CTTAACGTAGTCCTTGCAGG-3' | Andreas *et al.*, 1998 |
| Fatty acid binding protein 2 | *Hha* I RFLP | 5'-ACAGGTGTTAATATAGTGAAAAG-3'<br>5'-TACCCTGAGTTCAGTTCCGTC-3' | Kim *et al.*, 2001 |

**Table 2.** Genotype and allele frequencies of candidate gene polymorphisms in obese group and lean group

| | Genotypes | | | Allele Frequency | P value |
|---|---|---|---|---|---|
| | n (%) | n (%) | n (%) | | |
| $\alpha_{2A}$-AR C-1291G | CC | CG | GG | f (G) | |
| obese | 5 (17.2) | 24 (82.8) | 0 (0.0) | 0.59 | 0.93 |
| lean | 8 (13.3) | 52 (86.7) | 0 (0.0) | 0.43 | |
| $\beta_2$-AR T164I | TT | TI | II | f (I) | |
| obese | 29 (100.0) | 0 (0.0) | 0 (0.0) | 1.00 | 1.00 |
| lean | 59 (100.0) | 0 (0.0) | 0 (0.0) | 1.00 | |
| $\beta_3$-AR T64A | TT | TA | AA | f (A) | |
| obese | 21 (70.0) | 8 (26.7) | 1 (3.3) | 0.17 | 0.57 |
| lean | 43 (75.4) | 14 (24.6) | 0 (0.0) | 0.12 | |
| **LMNA C1908T[1]** | **CC** | **TC** | **TT** | **f (T)** | |
| **obese** | **13 (43.3)** | **15 (50.0)** | **2 (6.7)** | **0.32** | **0.88** |
| **lean** | **40 (65.6)** | **21 (34.4)** | **0 (0.0)** | **0.17** | |
| NPRC C-55A | CC | AC | AA | f (A) | |
| obese | 23 (100.0) | 0 (0.0) | 0 (0.0) | 1.00 | 0.48 |
| lean | 46 (97.9) | 1 (2.1) | 0 (0.0) | 0.99 | |
| **UCP1 A-3826G[2]** | **AA** | **AG** | **GG** | **f (G)** | |
| **obese** | **8 (21.6)** | **17 (45.9)** | **12 (32.4)** | **0.55** | **0.37** |
| **lean** | **16 (20.0)** | **51 (63.8)** | **13 (16.3)** | **0.48** | |
| Leptin C315T | CC | CT | TT | f (C) | |
| obese | 36 (100.0) | 0 (0.0) | 0 (0.0) | 1.00 | 1.00 |
| lean | 60 (100.0) | 0 (0.0) | 0 (0.0) | 1.00 | |
| FABP2 A54T | AA | AT | TT | f (T) | |
| obese | 10 (34.5) | 18 (62.1) | 1 (3.4) | 0.35 | 0.53 |
| lean | 20 (33.9) | 30 (50.8) | 9 (15.3) | 0.41 | |

[1]Statistically significant in dominant model ($\chi^2$=4.090, df=1, P=0.043).
[2]Statistically significant in recessive model ($\chi^2$=3.943, df=1, P=0.047).

The polymorphic patterns of each candidate genes were displayed in Table 2.

### $\alpha_{2A}$-adrenergic receptor gene

The $\alpha_{2A}$-adrenergic receptors meditate part of the actions of the catecholamines noradrenaline and adrenaline on the regulation of energy balance (Paula *et al.*, 1999). The human $\alpha_{2A}$-adrenergic receptor gene is located at chromosome 10q23-q25. The complete nucleotide sequence of this gene (HUMADRA2R) has been previously reported (Fraser *et al.*, 1989), and three restriction fragment length polymorphisms (*Dra* I, *Bsu*36 I, and *Msp* I RFLPs) have been reported to date (Hoehe *et al.*, 1988; Sergio *et al.*, 1997; Sun *et al.*, 1992). We have investigated a *Msp* I polymorphism of $\alpha_{2A}$-adrenergic receptor gene in Korean obese and lean group. The observed genotype frequencies of CC, CG and GG were 17.2%, 82.8% and 0.0% in obese group, and 13.3%, 86.7% and 0.0% in lean group, respectively. The GG genotypes was not observed in both groups. Frequencies of the G allele were 0.59 for obese group and 0.43 for lean group, respectively. There were no statistically significant differences between obese and lean groups in allele and genotype frequencies, respectively.

### $\beta_2$-adrenergic receptor gene

The human $\beta_2$-adrenergic receptor ($\beta_2$-AR) is a seven transmembrane G protein receptor found in vascular and adipose tissues. Stimulation of this receptor results in vasodilation (Dage *et al.*, 1983; Kirby *et al.*, 1991), and promotes lipolysis in human adipose tissue (Barbe *et al.*, 1996). Genetic variations at the $\beta_2$-adrenergic receptor gene locus have been associated with obesity and increased receptor sensitivity in women (Larger *et al.*, 1997). We have investigated a *Mnl* I polymorphism of $\beta_2$-adrenergic receptor gene in Korean obese and lean group. Only TT genotype was observed in both obese and lean group.

### $\beta_3$-adrenergic receptor gene

The $\beta_3$-adrenergic receptor is a seven membrane spanning protein which is expressed in visceral adipose

tissue, and is thought to regulate lipolysis and energy expenditure via thermogenesis (Revelli *et al.*, 1993). Studies in Pima Indians (Walston *et al.*, 1995), French Caucasians (Clement *et al.*, 1995), Finns (Widen *et al.*, 1995), Danes (Urhammer *et al.*, 1996), Japanese (Kadowaki *et al.*, 1995), and Australian Caucacians (Kurabayashi *et al.*, 1996) have shown modest associations between the Arg allele of *Bst* OI RFLP and various anthropometric markers of obesity and diabetes (Biery *et al.*, 1997; Proenza *et al.*, 2000). We have investigated a *Bst*O I polymorphism of $\beta_3$-adrenergic receptor gene in Korean obese and lean group. The observed genotype frequencies of TT, TA and AA were 70.0, 26.7 and 3.3% in obese group, and 75.4, 24.6 and 0.0% in lean group, respectively. The AA genotypes was only observed in obese group. Frequencies of the A allele were 0.17 for obese group and 0.12 for lean group, respectively. There were no statistically significant differences between obese and lean groups in allele and genotype frequencies, respectively.

### LMNA gene

Lamin A and C are ubiquitous structural proteins that polymerize in the nuclear lamina, a meshwork underlying the inner nuclear membranes, in which they interact with integral proteins and chromatin (Stuurman *et al.*, 1998). Recently, *Pml* I polymorphism was discovered in exon 10 of LMNA gene (Robert *et al.*, 2001). This polymorphism is namely a silent C → T substitution at nuceotide 1908, which is the last codon shared in common between lamin A and C before alternative splicing gives rise to the two distinct proteins. The studies in Inuit (Robert *et al.*, 2001), aboriginal Canadians (Robert *et al.*, 2000) have shown highly significant association of LMNA gene 1908T/T genotype with physical indeces of obesity. We have investigated a *Pml* I polymorphism of LMNA gene in Korean obese and lean group. The observed genotype frequencies of CC, CT and TT were 43.3, 50.0 and 6.7% in obese group, and 65.6, 34.4 and 0.0% in lean group, respectively. The TT genotypes was only observed in obese group. Frequencies of the T allele were 0.32 for obese group and 0.17 for lean group, respectively. There were the significant deviation from Hardy-Weinberg equilibrium in observed genotype frequencies. Because this C → T substitution of nucleotide 1908 of LMNA gene is a silent mutation, and have no effect on the protein structure of function of this gene, this deviation from Hardy-Weinberg equilibrium may not be due to natural selection. Therefore, founder effect might be operating in C → T substitution of LMNA gene. There were statistically significant differences between obese and lean groups in allele and genotype frequencies respectively. In dominant model, the odds ratio (95% CI) value of this polymorphism is 2.49 (1.02-6.09), and this value was statistically significant ($\chi^2$=4.090, df=1, P=0.043). However, considerable caution is needed in interpreting the statistical significance with the C → T substitution of LMNA gene observed in the present study by the shortage of sample size in this study, we can only set a limited potential value for our study. Further investigations are required into whether these findings are applicable to other ethnic groups. Therefore, we suggested the T allele at LMNA gene should be used as an available genetic marker for obesity diagnostics in Korean population.

### Clearance receptor gene

The clearance receptor for natriuretic peptides (NPRC) is highly expressed in adipose tissue, where is nutritionally regulated (Sarzani *et al.*, 1995). Moreover, in obese hypertensive patients, atrial natriuretic peptide (ANP) levels are reduced, the ratio of NPrA/NPrC is decreased in adipose tissue, and a low calorie diet can increase the biological effects of infused ANP (Dessi-Fulgheri *et al.*, 1997; Dessi-Fulgheri *et al.*, 1999). Recently, a *Hga* I polymorphism was discovered at position 55 of NPRC gene, and this genetic marker has been associated with lower atrial natriuretic peptide and higher blood pressure in obese hypertensives (Sarzani *et al.*, 1999). We have investigated a *Hga* I polymorphism of this gene in Korean obese and lean group. The observed genotype frequencies of CC, CA and AA were 100.0, 0.0, and 0.0% in obese group, and 97.9, 2.1 and 0.0% in lean group, respectively. The AA genotypes was not observed in two groups. Frequencies of the C allele were 1.00 for obese group and 0.99 for lean group, respectively. There were no statistically significant differences between obese and lean groups in allele and genotype frequencies respectively.

### Uncoupling protein 1 gene

In rodents, uncoupling protein 1 (UCP1) alters respiration coupling and dissipates oxidation energy as heat to maintain body temperature (Klaus *et al.*, 1991). Recent studies on human have suggested an association between the A to G substitution of the UCP1 gene and an increased capacity to gain weight (Clement *et al.*, 1996; Oppert *et*

*al.*, 1994), resistance to low calorie diet (Fumeron *et al.*, 1996) or synergistic effect in decreasing sympathetic nervous system activity with *Bst*O I polymorphism of $\beta_3$-adrenergic receptor gene (Shihara *et al.*, 2001). We have investigated a *Bcl* I polymorphism of UCP1 gene in Korean obese and lean group. The observed genotype frequencies of AA, AG and GG were 21.6, 45.9, and 32.4% in obese group, and 20.0, 63.8 and 16.3% in lean group, respectively. Frequencies of the G allele were 0.55 for obese group and 0.48 for lean group, respectively. There were no statistically significant differences between obese and lean groups in allele and genotype frequencies respectively. But, the odds ratio (95% CI) value of this polymorphism is 2.47 (1.00-6.14) in recessive model, and this value was statistically significant ($\chi^2$=3.943, df=1, P=0.047). Therefore, we suggested the UCP1 A allele should be used by a available genetic marker for obesity diagnostics in Korean population.

### Fatty acid binding protein 2 gene

Fatty acid binding proteins are intracellular proteins found in many tissues. They are involved in fatty acid transfer and metabolism, but their exact functions are not well known (Lowe *et al.*, 1987; Sweetser *et al.*, 1987). Fatty acid binding protein 2 (FABP2) expression is limited to the columnar absorptive epithelial cells of the small intestine (Cohn *et al.*, 1992; Sweetser *et al.*, 1987). This suggests that FABP2 should have a role in the absorption and intracellular transport of dietary long-chain fatty acids (Lowe *et al.*, 1987). Study in Pima Indians has shown the significant associations of FABP2 *Hha* I polymorphism with increasing lipid oxidation (Baier *et al.*, 1995). We have investigated a *Hha* I polymorphism of FABP2 gene in Korean obese and lean group. The observed genotype frequencies of AA, AT and TT were 34.5, 62.1, and 3.4% in obese group, and 33.9, 50.8 and 15.3% in lean group, respectively. Frequencies of the T allele were 0.35 for obese group and 0.41 for lean group, respectively. There were no statistically significant differences between obese and lean groups in allele and genotype frequencies.

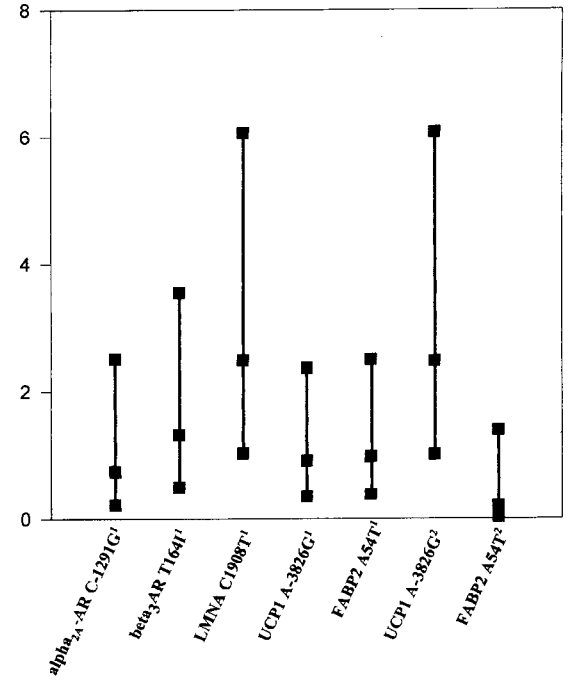


Fig. 1. Genotypic odds ratios for obesity and 95% confidence intervals, assuming a dominant[1] and recessive[2] genetic model. For all polymorphisms, the major allele was taken as the reference allele. For $beta_2$-AR T164I, NPRC C-55A, and Leptin C315t, the dominant model was not considered because of the low frequency of the minor allele. For $alpha_{2A}$-AR C-1291G, $beta_2$-AR T164I, $beta_3$-AR T164I, LMNA C1908T, NPRC C-55A, and Leptin C315T, the recessived model was not considered becaused of the same reason. In our example, disease allele is defined as allele with higher frequency in obese group compared with lean group.

**Table 3.** Odds ratio (OR) and 95% confidence interval (CI) values of polymorphisms in eight candidate genes

| Polymorphism | Causative allele | OR (95% CI) | |
|---|---|---|---|
| | | Dominant | Recessive |
| $\alpha_{2A}$-AR C-1291G | G | 0.74 (0.22-2.50) | – |
| $\beta_3$-AR T64A | A | 1.32 (0.49-3.53) | – |
| **LMNA C1908T** | **T** | **2.49 (1.02-6.09)**[1] | – |
| **UCP1 A-3826G** | **G** | 0.91 (0.35-2.36) | **2.47 (1.00-6.14)**[1] |
| FABP A54T | T | 0.97 (0.38-2.49) | 0.20 (0.02-1.65) |

The NPRC C-55A site showed the low frequency of minor allele, and $\beta_2$-AR T164I and leptin C315T sites were monomorphic. Therefore, the OR (95% CI) values could not calculated in our study.
[1]Statistically significant by $\chi^2$-test ($P < 0.05$).

### Leptin gene

Leptin is a adipose tissue-secreted hormone postulated to regulate energy intake, body adiposity and reproductive competence (Chehab *et al.*, 1996; Zhang *et al.*, 1994). Mutation in the mouse leptin gene lead to severe obesity (Andreas *et al.*, 1998). Aberrant secretion of leptin and deficient leptin receptor function have been shown to cause obesity in animal models (Ghilardi *et al.*, 1996; Lee *et al.*, 1996; Zhang *et al.*, 1994) and human (Clément *et al.*, 1998; Montague *et al.*, 1997). A leptin deficiency and receptor defects cause the same obese phenotype as in the genetically obese rodent models suggests a role for leptin in human obesity (Safak *et al.*, 1999). We have investigated a *Msp* I polymorphism of leptin gene in Korean obese and lean group. Both obese and lean group were only shown a CC genotype.

In Figure 1 and Table 3, we suggests both *Pml* I polymorphism of LMNA gene in dominant model and *Bcl* I polymorphism of UCP-1 gene in recessive model are shown to be associated with obesity in Koreans. Therefore, these two polymorphism may be useful as a genetic marker in obesity diagnostics in Koreans. There could be several reasons that might explain why the others were failed to show an association with obesity. First, the investigated gene, despite being strong candidates a priori, do not play any significant role in the pathogenesis of obesity. Secondly, the polymorphisms selected in each gene were not appropriate, or there may be exist other unmeasured polymorphisms of these genes whose effect on disease could not be detected through linkage disequilibrium with the polymorphisms studied. A third explanation might be related to the heterogeneity of patients with respect to progression of the disease.

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## References

Andreas, S., Tarik, I., Luc, C., Metin, O., and Strosberg, A. D. (1998): A mutation in PDS cause non-syndromic recessive deafness, *Nature Genet.* **18,** 213-215.

Baier, L. J., Sacchettini, J. C., and Knowler, W.C. (1995): An amino acid substitution in the human intestinal fatty acid binding protein associated with increased fatty acid binding, increased fat oxidation, and insulin resistance, *J. Clin. Invest.* **95,** 1281-1287.

Barbe, P., Millet, L., Galitzki, J., Lafontan, M., and Berlan, M. (1996): *In situ* assessment of the role of â$_1$-, â$_2$-, and â$_3$-adrenoceptors in the control of lipolysis and nutritive blood flow in human subcutaneous adipose tissue, *Br. J. Pharmacol.* **117,** 907-913.

Biery, A. J., Ebbesson, S. O. E., Shuldiner, A. R., and Boyer, B. B. (1997): The â$_3$-adrenergic receptor TRP64ARG polymorphism and obesity in Alaskan Eskimos, *Int. J. Obes. Relat. Metab. Disord.* **21,** 1176-1179.

Bouchard, C., Pérusse, L., Leblanc, C., Tremblay, A., and Theriaut, G. (1998): Inheritance of the amount and distribution of human body fat, *Int. J. Obes. Relat. Metab. Disord.* **12,** 205-215.

Cambien, F., Poirier, O., Mallet, C., and Tiret, L. (1997): Coronary heart disease and genetics: an epidemiologists view, *Mol. Med. Today.* **3,** 197-202.

Chehab, F. F., Lim, M. E., and Lu, R. (1996): Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin, *Nature Genet.* **12,** 318-320.

Clement, K., Ruiz, J., and Cassard-Doucier, A. M. (1996): Additive effect of A?G(-3826) varient of the uncoupling protein gene and the Trp64Arg mutation of the â$_3$-adrenergic receptor gene on weight gain in morbid obesity, *Int. J. Obes. Relat. Metab. Disord.* **20,** 1062-1066.

Clement, K., Vaisse, C., Manning, B. S., Basdevant, A., Guy-Grand, B., Ruiz, J., Silver, K. D., Shuldiner, A. R., Froguel, P., and Strosberg, A. D. (1995): Genetic variation in the beta(3)-adrenergic-receptor and an inceased capacity to gain weight in patients with morbid obesity, *N. Engl. J. Med.* **333,** 352-354.

Clément, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M., Dina, C., Chambaz, J., Lacorte, J. M., Basdevant, A., Bougnères, P., Lebouc, Y., Froguel, P., and Guy-Grand, B. (1998): A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction, *Nature* **392,** 398-401.

Cohn, S. M., Simon, T. C., and Roth, K. A. (1992): Use of transgenic mice to map cis-acting elements in the intestinal fatty acid binding protein gene that control its cell lineage-specific and regional patterns of expression along the duodenal-colonic and crypt-villus axes of the gut epithelium, *J. Cell. Biol.* **119,** 27-44.

Dage, R. C., Hsieh, C. P., and Spedding, M. (1983): Vasodilation by medroxalol mediated by â$_3$-adrenergic receptor stimulation, *J. Cardiovasc. Pharmacol.* **5,** 143-150.

Dessi-Fulgheri, P., Sarzani, R., Tamburrini, P., Moraca, A., Espinosa, E., and Cola, G. (1997): Plasma aritial natriuretic peptide and natriuretic peptide receptor gene expression in adipose tissue of normotensive and hypertensive obese patients, *J. hypertens.* **15,** 1695-1699.

Dessi-Fulgheri, P., Sarzani, R., Serenelli, M., Tamburrini, P.,

Spagnolo, D., and Gaintomassi, L. (1999): Low calorie diet enhances renal, hemodynamic, and humoral effects of exogenous atrial natriuretic peptide in obese hypertensives, *Hypertension* **33,** 658-662.

Fraser, C. M., Arakawa, S., McCombie, W. R., and Venter, J. C. (1989): Cloning, sequencing analysis and permanent expression of a human alpha2-adrenergic receptor in Chinese hamster ovary cells, *J. Biol. Chem.* **264,** 11754-11761.

Fumeron, F., Durack-Bown, I., and Betoulle, D. (1996): Polymorphism of the uncoupling protein (UCP) and the â$_3$-adrenergic receptor gene in obese people submitted to a low calorie diet, *Int. J. Obes. Relat. Metab. Disord.* **20,** 1051-1054.

Ghilardi, N., Ziegler, S., Wiestner, A., Stoffel, R., Heim, M. H., and Skoda, R. C. (1996): Defective STAT signaling by the leptin receptor in diabetic mice, *Proc. Natl. Acad. Sci. USA.* **93,** 6231-6535.

Herzog, H., Selbie, L.A., Zee, R. Y. L., Morris, B. J., and Shine, J. (1993): Neuropeptide-Y Y1 receptor gene polymorphism: cross-sectional analysis in essential hypertension and obesity, *Biochem. Biophys. Res. Commun.* **196,** 902-906.

Hoehe, M. R., Berrettini, W. H., and Lentes, K. U. (1988): *Dra* I identifies a two allele DNA polymorphism in the human alpha-2 adrenergic receptor gene (ADRAR), using a 5.5 kb probe, *Nucleic. Acids. Res.* **16,** 9070.

Kadowaki, H., Yasuda, K., and Iwamoto, K. (1995): A mutation in the beta(3)-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japan subjects, *Biochem. Biophys. Res. Commun.* **215,** 555-560.

Kim, C. H., Yun, S. K., Byun, D. W., Yoo, M. H., Lee, K. U., and Suh, K. I. (2001): Codon 54 polymorphism of the fatty acid binding protein 2 gene is associated with increased fat oxidation and hyperinsulinemia, but not with intestinal fatty cid absorption in Korean men, *Metabolism* **50,** 473-476.

Kirby, R. F., Woodworth, C. H., Woodworth, G. G., and Johnson, A. K. (1991): $\beta_2$-adrenoceptor mediated vasodilation: role in cardiovascular responses to acute stressors in spontaneously hypertensive rats, *Clin. Exp. Hypertens.* **13,** 1059-1068.

Kissebah, A. H., Freedman, D. S., and Peiris, A. N. (1989): Health risks of obesity, *Med. Clin. N. Am.* **73,** 111-138.

Klaus, S., Casteilla, L., Bouillaud, F., and Ricquir, D. (1991): The uncoupling protein UCP: a membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue, *Int. J. Biochem.* **23,** 791-801.

Kristi, S., Braxton, D. M., Jeremy, W., John, D. S., Michael, P. S., Jesse, R., and Alan, R. S. (1997): TRP64ARG $\beta_3$-adrenergic receptor and obesity in Mexican Americans, *Hum. Genet.* **101,** 306-311.

Kurabayashi, T., Carey, D. G. P., and Morrison, N. A. (1996): The $\beta_3$-adrenergic receptor Trp64Arg mutation is overrepresented in obese women. *Diabetes* **45,** 1358-1362.

Lander, E. S. (1996): The new genomics: global views of biology, *Science* **274,** 536-539.

Larger, V., Hellström, L., Reynisdottir, S., Lõnnqvist, F., Eriksson, P., Lannfelt, L., and Arner, P. (1997) Human â$_2$-adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte â$_2$-adrenoceptor function, *J. Clin. Invest.* **100,** 3005-3013.

Lee, G. H., Proenca, R., Montez, J. M., Carroll, K. M, Darvishzadeh, J. G, Lee, J. I., and Friedman, J. M. (1996): Abnormal splicing of the leptin receptor in diabetic mice, *Nature* **379,** 632-635.

Lowe, J. B., Sacchettini, J. C., and Laposata, M. (1987): Expression of rat intestinal fatty acid-binding protein in Escherichia coli: Purification and comparision of ligand binding characteristics with that of Escherichia coli-derived rat liver fatty acid-binding protein, *J. Biol. Chem.* **262,** 5931-5937.

Montague, C.T., Prins, J.B., Sanders, L., Digby, J.E., and ORahilly, S. (1997): Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution, *Diabetes* **46,** 342-347.

Oppert, J. M., Vohl, M. C., and Chagnon, M. (1994): DNA polymorphism in the uncoupling protein (UCP) gene and human body fat, Int. *J. Obes. Relat. Metab. Disord.* **18,** 526-531.

Paula, H., Markku, K., Ullamari, P., Matti, K. K., Aila, R., Markku, L., Raisa, V., Matti, U., and Mika, S. (1999): Identification of a three-amino acid deletion in the â$_{2A}$-adrenergic receptor that is associated with reduced basal metabolic rate in obese subjects, *J. Clin. Endocrinol. Metab.* **84,** 2429-2433.

Proenza, A. M., Poissonnet, C. M., Ozata, M., Ozen, S., Guran, S., Palou, A., and Strosberg, A. D. (2000): Association of sets of alleles of genes encoding beta3-adrenoreceptor, uncoupling protein 1 and lipoprotein lipase with increased risk of metabolic complications in obesity, *Int. J. Obes. Relat. Metab. Disord.* **24,** 93-100.

Revelli, J. P., Muzzin, P., and Paoloni, A. (1993): Expression of the $\beta_3$-adrenergic receptor in white adipose tissue, *J. Mol. Endocrinol.* **10,** 193-197.

Robert, A. H., Henian, C., Stewart, B. H., Bernard, Z., Anthony, J. H., and Carol, M. A. (2000): Genetic variation in LMNA modulates plasma leptin and indices of obesity in aboriginal Canadians, *Physiol. Genomics* **3,** 39-44.

Robert, A. H., Murray, W. H., and Young, T. K. (2001): Common genomic variation in LMNA modulated indexes of obesity in inuit, *J. Clin. Endocrinal. Metab.* **86,** 2747-2751.

Rutherford, S., Nyholt, D. R., Curtain, R. P., Quinlan, S. R., Gaffney, P. T., Morris, B. J., Griffiths, L. R. (1997): Association of a low density lipoprotein receptor microsatellite variant with obesity, *Int. J. Obes. Relat. Metab. Disord.* **21,** 1032-1037.

Safak, G., Ahmed, E. B., Gabriele, E. S., Charles, R. W., Raymond, G. H., Glenn, R. K., and Ahmed, H.K. (1999):

Plasma leptin and insulin levels in weight-reduced obese women with normal body mass index, *Diabetes* **48,** 347-352.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989): Molecular cloning: a Laboratory Manual. 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 9.16-9.23.

Sarzani, R., Dessi-Fulgheri, P., Salvi, F., Serenelli, M., Spagnolo, D., Cola, G., Pupita, M., Giantomassi, L. and Rappelli, A. (1999): A novel promoter variant of the natriuretic peptide clearance receptor gene is associated with lower atrial natriuretic peptide and higher blood pressure in obese hypertensives, *J. Hypertens.* **17,** 1301-1305.

Sarzani, R., Paci, M. V., Zingaretti, M. C., Pierleoni, C., Cinti, S., and Cola, G. (1995): Fasting inhibits natriuretic peptides clearance receptor expression in rat adipose tissue, *J. Hypertens.* **13,** 1241-1246.

Sergio, L., Jordi, C., Aleix, C., Josep, O., Albert, T., and Francisca, R. (1997); *Msp* I identifies a biallelic polymorphism in the promoter region of the á$_{2A}$-adrenergic receptor gene, *Clin. Genet.* **51,** 129-130.

Shihara, N., Yasuda, K., Moritani, T., Ue, H., Uno, M., Adachi, T., Nunoi, K., Seino, Y., Yamada, Y., and Tsuda, K. (2001): Synergistic effect of polymorphisms of uncoupling protein 1 and beta3-adrenergic receptor genes on autonomic nervous system activity, *Int. J. Obes. Relat. Metab. Disord.* **25,** 761-766.

Stuurman, N., Heins, S., and Aebi, U. (1998): Nuclear lamins: their structure,assembly,and interactions, *J. Struct. Biol.* **122,** 42-66.

Sun, L., Schulte, N., Pettinger, P., Regan, J. W., and Pettinger, W. A. (1992): The frequency of $\alpha_2$-adrenoceptor restriction fragment length polymorphisms in normotensive and hypertensive humans, *J. Hypertens.* **10,** 1011-1015.

Sweetser, D. A., Birkenmeier, E. H., and Klisak, I. J. (1987): The human and rodent intestinal fatty acid binding protein genes: A comparative analysis of their structure, expression, and linkage relationships, *J. Biol. Chem.* **263,** 16060-16071.

Valve, R., Heikkinen, S., Rissanen, A., Laakso, M., and Uusitupa (1998): Synergistic effect of polymorphisms in uncoupling protein 1 and $\beta_3$-adrenergic receptor genes on basal metabolic rate in obese Finns, *Diabetologia* **41,** 357-361.

Walston, J., Silver, K., Bogardus, C., Knowler, W. C., Celi, F. S., Austin, S., Manning, B., Strosberg, A. D., Stern, M. P., Raben, N., Sorkin, J. D., Roth, J., and Shuldiner, A. R. (1995): Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the beta(3)-adrenergic-receptor gene, *N. Engl. J. Med.* **333,** 343-347.

Widen, E., Lehto, M., Kanninen, T., Walston, J., Shuldiner, A. R., and Groop, L. C. (1995): Association of a polymorphism in the beta(3)-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns, *N. Engl. J. Med.* **333,** 348-351.

Urhammeret, S. O., Clausen, J. O., Hansen, T., and Pedersen, O. (1996): Insulin sensitivity and body weight changes in young white carriers of the codon 64 amino acid polymorphism of the â$_3$-adrenergic receptor gene, *Diabetes* **45,** 1115-1120.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994): Positional cloning of the mouse obese gene and its human homologue, *Nature* **372,** 425-432.

Zee, R. Y. L., Schrader, A. P., Robinson, B. G., Griffiths, L. R. and Morris, B. J. (1995): Association of *Hinc* II RFLP of low density lipoprotein receptor gene with obesity in essential hypertensives, *Clin. Genet.* **47,** 118-121.

Zee, R. Y. L., Stephan, A. L., Iwai, N. and Morris, B. J. (1997): Association analysis of SA gene variant in essential hypertensives, *Am. J. Hypertens.,* **10,** 235-242.