Association between \textit{ESRI} rs1884051 polymorphism and dietary total energy and plant protein intake on obesity in Korean men

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Abstract

\textit{ESRI} has been listed in the Human Obesity Gene Map as candidate gene associated with obesity. Thus, in this study, we investigated the effect of the \textit{ESRI} rs1884051 polymorphism on obesity-related variables, together with their modulations by dietary intake in Korean men. The obesity-related variables and dietary intake of 3,039 Korean men aged 40-59 years from KoGES database were analyzed. Body weight ($P=0.007$), BMI ($P=0.003$), waist-hip ratio ($P=0.011$), fat body mass ($P=0.010$), and body fat percentage ($P=0.040$) were significantly lower in subjects with the minor T allele of \textit{ESRI} rs1884051 than in subjects carrying the C allele. Moreover, the rs1884051 T allele was associated with a decreased risk of obesity prevalence ($P=0.040$). Among the subjects whose total energy intake was below the median, carrier of the minor T allele of \textit{ESRI} rs1884051 had a lower BMI ($P=0.003$) when compared with subjects carrying the C allele. In addition, among subjects whose plant protein intake was above the median, carrier of the minor T allele of \textit{ESRI} rs1884051 had a lower BMI ($P=0.044$) compared with subjects carrying the C allele. Our findings demonstrate that there is a significant association between the \textit{ESRI} rs1884051 variant and obesity-related variables and this association can be potentially modified by dietary energy and plant protein intake.

Key Words: \textit{ESRI} gene, polymorphism, BMI, obesity, dietary intake

Introduction

Obesity is a major health concern worldwide and predisposes subjects to a high risk of premature mortality through an increased risk of many chronic diseases, including type 2 diabetes, cardiovascular diseases, metabolic syndromes, and cancers [1,2]. The primary cause of obesity is known as an accumulation of excessive body fat resulting from an imbalance of energy intake over physical activity [3]. However, genetic variations have recently been estimated to account for 40-70% of the population variance in obesity [4,5]. In other words, obesity is regarded as a complex multifactorial disease, which is affected by genetic variation as well as environmental factors, especially diet. Genetic variations may influence on individuals to show different predispositions over obesity through controlling a balance between energy intake and expenditure [6,7].

Estrogen and its receptors are essential for sexual development and reproductive function, but also play a role in protection against obesity and regulation of food intake [8,9]. The estrogen receptor 1 (\textit{ESRI}) gene encodes an estrogen receptor, which is a ligand-activated transcription factor composed of several important domains for hormone binding, DNA binding, and activation of transcription [9].

Recently the \textit{ESRI} has been listed in the Human Obesity Gene Map as candidate gene associated with obesity [10]. The \textit{ESRI} was expressed primarily by adipocytes and it attenuates the lipolytic action in subcutaneous fat through up-regulation of the number of antilipolytic alpha 2A-adrenergic receptors [11]. \textit{ESRI} polymorphisms have been reported to be associated with obesity-related variables, such as BMI and waist circumference [12-15].

It was reported earlier that rs9340799 (\textit{XbaI} in intron 1) of \textit{ESRI} was related with waist circumference and BMI in middle aged Japanese women but not in men [12]. In the Framingham Heart Study (FHS), \textit{ESRI} polymorphisms, rs223469 (\textit{PvuII} in intron 1) and rs9340799 (\textit{XbaI} in intron 1) were associated with waist circumference and rs1801132 (C1335G in exon 4) was associated with BMI in men, but not in women [13]. However, rs2234693 and rs9340799 of \textit{ESRI} were not associated with obesity in Swedish women [14] and Chinese population [15]. These inconsistencies might be explained by the differences in \textit{ESRI} from races and sex-dependent genetic contributions; some of these variations might correspond to functionally important mutations, but those found in other ethnic and gender groups might not. Furthermore, the interaction between genetic variation and...
dietary intake might affect the phenotype, which might be the source of the inconsistent results observed in these previous studies.

Therefore, we investigated the influence of \textit{ESR1} rs1884051 genotype on obesity-related phenotypes in the middle-aged Korean men, who participated in the Korean Genome Epidemiology Study (KoGES). We also evaluated the interaction between the \textit{ESR1} rs1884051 genotype and dietary intake in relation with body mass index (BMI).

**Subjects and Methods**

**Subjects**

3,039 Korean men aged between 40 and 59 years who participated in the Korean Genome Epidemiology Study (KoGES) in 2001 were used in this study [16]. The KoGES was performed as a cohort study to investigate chronic diseases (diabetes, hypertension, osteoporosis, obesity and metabolic syndrome) in adults aged 40-69 years old. The subjects were recruited from two community-based epidemiological studies in the rural Ansan and urban Ansan communities from 2001 to 2008. All subjects were of Korean ancestry. Among the 10,038 subjects in the KoGES, 3,039 men that were between 40 and 59 years old and had completed the dietary analysis were selected. This protocol used in this study was approved by the Institutional Review Board of Ewha Womans University, Seoul, South Korea.

**Anthropometric variables**

Body weight, height, and waist circumference were measured using a standardized procedure: height without shoes was measured using an anthropometer, weight in light clothes was determined using a weighting scale, and waist circumference over the unclothed abdomen at a minimal diameter was measured using non-stretchable standard tape. Body composition (lean body mass, fat body mass, body fat percentage, and waist/hip ratio) was measured using an In-body 3.0 (Biospace Co., Ltd, Seoul, Korea). BMI was calculated by dividing the weight in kilograms by the height in meters squared. Obesity was defined as BMI ≥ 25 kg/m² and body fat percentage > 25%, which was based on the WHO Asia-Pacific Area criterion for obesity [17].

**Blood biochemical measurements**

Venous blood was collected after an overnight fast, and all plasma samples were subjected to biochemical measurements. Fasting glucose, fasting insulin, total cholesterol, HDL cholesterol, and triglycerides were measured using a Hitachi 7600 Automatic Analyzer (Hitachi, Tokyo, Japan). The level of LDL cholesterol was calculated using the following equation, described by Friedewald, for individuals with a plasma triglyceride levels < 400 mg/dl [18]: LDL-cholesterol = [Total cholesterol - {HDL-cholesterol - (Triglycerides/5)}].

**Dietary analysis**

Dietary intake was estimated through a food frequency questionnaire (FFQ) that was developed and validated in the KoGES [19]. The correlation coefficients of nutrient intakes between the FFQ at the end of the study and the 12-day diet records showed 0.43 (protein), 0.40 (fat), and 0.64 (carbohydrate) for age, sex, de-attenuated and energy-adjusted data. The questionnaire consisted of questions on 103 food items, which were combined into the 23 nutrients used in the Korean food composition table. The data was analyzed to give an average daily dietary intake using the Computer-Aided Nutritional Analysis Program (CAN Pro) 3.0, which is a nutrient database developed by the Korean Nutrition Society [20]. The protein (total, animal, and plant), fat (total, animal, and plant), and carbohydrate intake were given as percentages of the total daily energy intake.

**Genotyping and SNP selection**

Genomic DNA was extracted from whole blood and genotyped on an Affymetrix Genome-Wide Human SNP Array 5.0 [16]. Among 39 SNPs in and around \textit{ESR1} were found from the KoGES, 10 SNPs were first eliminated if they deviated from Hardy-Weinberg equilibrium \((P<0.05)\). Then, SNPs with minor allele frequencies below 0.05 were eliminated in the analysis. Lastly, \textit{ESR1} rs1884051, which presented the significant associations with obesity-related phenotypes, was studied.

**Statistical analysis**

All statistical analyses were performed using SPSS provided in the Windows software (version 17.0; SPSS Inc, Chicago, IL) with a level of significance at \(P<0.05\). Allele frequencies were calculated by allele counting and the departure from Hardy-Weinberg equilibrium was calculated using the chi-square test. To determine the most appropriate model, we tested different genetic models (dominant, additive and recessive). A recessive model, which carriers of 1 or 2 copies of the major allele (n = 2,387) were grouped and compared with minor allele homoygotes (n = 652), was used. Data for continuous variables was presented as mean values ± SEM (standard error of mean). Generalized linear models were applied to compare variables on the basis of the \textit{ESR1} rs1884051 genotype after adjustment for covariates. Potential confounding variables were either statistically significant in univariate analyses or known to be potentially important factors related to genotype or BMI, such as age, hypertension, diabetes, thyroid gland disease, dyslipidemia, smoking, and alcohol consumption. The effect of \textit{ESR1}
rs1884051 genotype on the risk of obesity (as BMI and body fat percentage) was examined using logistic regression analysis, after adjustment for covariates, to estimate the odds ratios (ORs) and 95% confidence intervals (CI). To determine the interaction between the ESR1 rs1884051 genotype and intake of macronutrients, subjects were categorized by comparing their level of total energy intake and the percentage of energy they obtained from protein (total, animal, and plant), fat (total, animal, and plant), and carbohydrate with the median levels for these parameters. A multivariate interaction model was used to evaluate the interactions between the ESR1 rs1884051 genotype and dietary macronutrient intake, and their effect on BMI after adjustment for covariates.

Results

Anthropometric and biochemical characteristics

The anthropometric and biochemical characteristics of the 3,039 subjects are listed in Table 1.

In the recessive model, the ESR1 rs1884051 polymorphism was significantly associated with the obesity-related variables, after adjustment for age, hypertension, diabetes, thyroid gland disease, dyslipidemia, smoking, and alcohol consumption (Fig. 1). No association between ESR1 rs1884051 genotype and the prevalence of obesity, which was defined as BMI ≥ 25 kg/m², was observed (CC+TC 24.62 ± 0.06 kg/m² vs. TT 24.24 ± 0.11 kg/m², P = 0.003), waist-hip ratio (CC+TC 0.90 ± 0.00 vs. TT 0.89 ± 0.00, P = 0.012), fat body mass (CC+TC 15.53 ± 0.10 kg 14.96 ± 0.25 kg, P = 0.011), and body fat percentage (CC+TC 21.88 ± 0.10% vs. TT 21.42 ± 0.20%, P = 0.041) when compared to those carrying the C allele.

The biochemical characteristics, such as the plasma level of glucose, insulin, cholesterol (total, HDL, and LDL), and triglycerides, of the subjects did not differ among the ESR1 rs1884051 polymorphism (Table 1).

The genotype distribution of SNP in ESR1 rs1884051 was in the Hardy-Weinberg equilibrium (P = 0.28), and the minor allele frequency (MAF) of polymorphism in ESR1 rs182052 was 0.47 (Table 1).

Table 1. Anthropometric data and blood profiles of subjects with the ESR1 rs1884051 genotype in Korean men

| Parameter                  | CC+TC (n = 2,387) | TT (n = 652) | P-value |
|----------------------------|-------------------|-------------|---------|
| Height (cm)                | 167.87 ± 0.11     | 167.89 ± 0.22 | 0.469   |
| Weight (kg)                | 69.52 ± 0.19      | 68.49 ± 0.36 | 0.011   |
| BMI (kg/m²)                | 24.62 ± 0.11      | 24.24 ± 0.11 | 0.003   |
| Waist circumference (cm)   | 83.80 ± 0.16      | 84.02 ± 0.30 | 0.524   |
| Waist-hip ratio            | 0.90 ± 0.00       | 0.89 ± 0.00  | 0.012   |
| Lean body mass (kg)        | 53.26 ± 0.13      | 53.73 ± 0.25 | 0.055   |
| Fat body mass (kg)         | 15.53 ± 0.10      | 14.96 ± 0.25 | 0.011   |
| Body fat percentage (%)    | 21.88 ± 0.10      | 21.42 ± 0.20 | 0.041   |
| Obesity prevalence (n (%)) |                   |             |         |
| BMI ≥ 25 kg/m²             | 1,050 (44.0)      | 277 (42.5)  | 0.504   |
| Body fat percentage ≥ 25%  | 543 (26.9)        | 123 (22.5)  | 0.042   |
| Glucose (mg/dl)            | 90.11 ± 0.53      | 89.87 ± 1.01 | 0.912   |
| Insulin (μU/ml)            | 7.17 ± 0.08       | 7.12 ± 0.16  | 0.753   |
| Total cholesterol (mg/dl)  | 195.10 ± 0.73     | 194.83 ± 1.41 | 0.917  |
| HDL-cholesterol (mg/dl)    | 43.16 ± 0.20      | 43.28 ± 0.38 | 0.768   |
| LDL-cholesterol (mg/dl)    | 115.22 ± 0.73     | 115.09 ± 1.39 | 0.933   |
| Triglycerides (mg/dl)      | 183.10 ± 2.58     | 182.33 ± 5.00 | 0.892   |

Effect of the ESR1 rs1884051 genotype on the risk of obesity prevalence

We analyzed whether the ESR1 rs1884051 genotype affected the risk of obesity prevalence based on different criteria for obesity using logistic regression analysis after adjustment for age, hypertension, diabetes, thyroid gland disease, dyslipidemia, smoking, and alcohol consumption. The ESR1 rs1884051 genotype on the risk of obesity prevalence was not detected (Fig. 1). When obesity prevalence was defined as body fat percentage > 25%, the prevalence of obesity was significantly lower in subjects with the homozygous T allele of ESR1 rs1884051 than in those carrying the C allele, CC+TC: 26.9% and TT: 22.5% respectively, after adjustment for covariates (Table 1). Moreover, the subjects with the minor T allele of ESR1 rs1884051 had a significantly decreased risk of obesity than those carrying the C allele (OR = 0.79, 95% CI = 0.63-0.99, P = 0.041, Fig. 1).

![Fig. 1. Odd Ratio (OR) and 95% confidence interval (95% CI) for obesity based on obesity-related phenotypes (BMI and body fat percentage) of subject with the ESR1 rs1884051 genotype in Korean men. Odds ratios (95% CI) in the aspect of the risk allele using a recessive model (CC+TC vs. TT). Cutoffs for obesity were based on WHO Asia-Pacific Area criteria for obesity (BMI ≥ 25 kg/m² and body fat percentage > 25%).](image-url)
The effects of interaction between the ESR1 rs1884051 genotype and dietary intake on BMI

Dietary intake was analyzed to examine whether the ESR1 rs1884051 genotype affected dietary macronutrient intake (Table 2). The polymorphism of ESR1 rs1884051 was not associated with the mean values of the total energy intake. Similarly, the percentage of energy obtained from protein (total, animal, and plant), fat (total, animal, and plant), and carbohydrate consumption were independent of the ESR1 rs1884051 genotype.

We analyzed whether the interaction between the ESR1 rs1884051 genotype and dietary macronutrient intake affected the BMI (Table 3). We sorted each subject by comparing their total energy intake and the percentage of energy they obtained from Table 2. Dietary intake of subjects with the ESR1 rs1884051 genotype in Korean men

|                        | CC+TC (n = 2,387) | TT (n = 652) | P-value |
|------------------------|-------------------|-------------|---------|
| Energy (kcal)          | 2,147.42 ± 11.52  | 2,120.36 ± 22.06 | 0.277   |
| Protein (%E)           | 15.77 ± 0.05      | 15.84 ± 0.09 | 0.481   |
| Animal (%E)            | 6.27 ± 0.05       | 6.34 ± 0.10 | 0.523   |
| Plant (%E)             | 9.40 ± 0.03       | 9.51 ± 0.06 | 0.764   |
| Fat (%E)               | 20.51 ± 0.10      | 20.57 ± 0.20 | 0.810   |
| Animal (%E)            | 10.10 ± 0.09      | 10.37 ± 0.17 | 0.613   |
| Plant (%E)             | 9.76 ± 0.06       | 9.93 ± 0.11 | 0.163   |
| Carbohydrate (%E)      | 64.16 ± 0.14      | 63.96 ± 0.26 | 0.483   |

The data represent the means ± SEM and P values after adjustment for age, hypertension, diabetes, thyroid gland disease, dyslipidemia, smoking, and alcohol consumption.

Table 3. The effects of the interaction between the ESR1 rs1884051 genotype and dietary intake on BMI in Korean men

|                        | CC+TC (n = 2,387) | TT (n = 652) | P-value* | P-value** |
|------------------------|-------------------|-------------|----------|-----------|
| Energy (kcal)          | < 2,053.24        | ≥ 2,053.24  | 0.001    | 0.003     |
| Protein (%E)           | < 15.80           | ≥ 15.80     | 0.006    | 0.248     |
| Animal protein (%E)    | < 6.09            | ≥ 6.09      | 0.001    | 0.326     |
| Plant protein (%E)     | < 9.38            | ≥ 9.38      | 0.001    | 0.001     |
| Fat (%E)               | < 20.60           | ≥ 20.60     | 0.003    | 0.205     |
| Animal fat (%E)        | < 9.85            | ≥ 9.85      | 0.003    | 0.161     |
| Plant fat (%E)         | < 9.74            | ≥ 9.74      | 0.008    | 0.201     |
| Carbohydrate (%E)      | > 64.10           | ≥ 64.10     | 0.179    | 0.296     |

The data represent the means ± SEM and P values after adjustment for age, hypertension, diabetes, thyroid gland disease, dyslipidemia, smoking, and alcohol consumption.

Discussion

ESR1 on chromosome 6q25 is comprised of 8 exons and 7 introns with a total size of 140 kilobases [4]. The ESR1 rs1884051 polymorphism occurs because of the transversion of C to T at nucleotide 271749 (NM_001122742) in intron 4 of the ESR1 gene, which does not result in amino acid changes. This MAF was different from that observed in a European population (29.7%), but was similar to that observed in other Asian populations (Japanese: 48.9% and Chinese: 45.6%) and in an African (41.7%) population obtained from HapMap data.

In this study, significant associations between obesity-related variables and ESR1 rs1884051 polymorphism were identified. Subjects with the homozygous minor T allele of ESR1 rs1884051 showed significantly lower body weight (P = 0.011), BMI (P = 0.003), waist-hip ratio (P = 0.012), fat body mass (P = 0.011), and body fat percentage (P = 0.041) when compared to subjects carrying the major C allele after adjustment for age, hypertension, diabetes, thyroid gland disease, dyslipidemia, smoking, and alcohol consumption. These results were consistent with earlier studies that examined other ESR1 polymorphisms [12,13,21]. For example, Okura et al. [12] found a similar association with obesity-related variables and the rs9340799 (XbaI) polymorphism in 2,238 Japanese subjects. Older women (60-79 years) with homozygous minor G allele had smaller fat mass by 18% and waist circumference by 6% when compared to those carrying the homozygous major A allele. On the other hand, no difference was found among the rs9340799 genotype for men. The
Framingham Heart Study (FHS) investigated the association between the \textit{ESR1} polymorphisms and BMI or waist circumference in 1,763 unrelated men and women [13]. In this population, men with the minor homozygous allele for the rs2224693 (PvuII) and rs9340799 (XbaI) had lower waist circumference than those with the major allele. Also, subjects with the minor allele for the rs1801132 (C1353G) had a lower BMI when compared to subjects with the major allele in men, but not women. In addition, Gallagher et al. [21] reported that among 17 \textit{ESR1} polymorphisms, rs6902771, rs2431260 and rs2175898 were associated with BMI in African Americans from the Insulin Resistance Atherosclerosis Family Study. Our results provide a new perspective in studying associations between genetic variation of \textit{ESR1} rs2175898 and the obesity in middle-aged Korean men.

Our result showed that the prevalence of obesity, which is defined as body fat percentage > 25%, was significantly lower in subjects with the minor T allele of \textit{ESR1} rs1884051 than in subjects carrying the major C allele. Moreover, subjects with homozygous minor T allele had a lower risk of obesity by 79%, when compared to individuals carrying the C allele. Recently, Goulart et al. [22] showed that women with the variant of rs2234693 had a lower prevalence of obesity when compared to those with the wild type allele and the rs2234693 polymorphism was inversely associated with risk of obesity, which was in agreement with our results. These results imply that there is a decrease in the prevalence and risk of obesity in subjects with the \textit{ESR1} rs1884051 T allele.

We also analyzed the effect of genetic variations on dietary macronutrient intake. No significant differences in dietary intake of energy and the percentage of energy they obtained from protein (total, animal, and plant), fat (total, animal, and plant), and carbohydrate were observed among subjects with different genotypes of \textit{ESR1} rs1884051. These results suggested that genetic variations in \textit{ESR1} rs1884051 might not influence dietary macronutrient intake.

In our study, a significant novel interaction between \textit{ESR1} rs1884051 genotype and dietary intake of energy with respect to BMI was identified. Among subjects with low energy intake, carriers of the minor T allele of \textit{ESR1} rs1884051 had a lower BMI than subjects carrying the C allele, whereas for subjects with a high energy intake, the \textit{ESR1} rs1884051 polymorphism did not affect BMI. Miyaki et al. [23] found that dietary energy intake affected the association between the Trp64Arg polymorphism of beta 3-adrenergic receptor (\textit{ADRB3}) and obesity in 295 healthy Japanese men. Among the subjects who had a high energy intake, the \textit{ADRB3} variant with respect to BMI in future studies.

In conclusion, this study demonstrated that the genetic variation in \textit{ESR1} rs1884051 was directly associated with obesity-related variables such as body weight, BMI, waist-hip ratio, fat body mass, and body fat percentage in Korean men. Moreover, the risk of obesity was also affected by variations in \textit{ESR1} rs1884051. In particular, with respect to the intake of a low level of dietary energy and high level of dietary plant protein, subjects with the minor T allele of \textit{ESR1} rs1884051 had significantly lower BMI than those carrying the C allele. Although these findings will need to be reproduced in further studies, this is the first study to demonstrate the concept of using a personalized approach to control obesity and prevent disease.

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