보건학 석사학위 논문

Genome-wide association study on eating behavior measured by Dutch Eating Behavior Questionnaire (DEBQ)

식이행동 관련 유전 변이의 탐색

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초록

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연구목적. 한국인 집단에서 식이행동 문제를 기인하는 특정 유전요인을 탐색하고자 한다.

연구방법. 연구대상자는 한국인 가족-쌍둥이 코호트 연구에 참여한 대상자 중 DEBQ설문에 응답하고 비만 수준이 조사된 2606명으로 한다. 식이행동는 Dutch Eating Behavior Questionnaire (DEBQ)로 조사되었으며, 본 설문은 세 가지의 식이행동를 조사하는 33개의 항목으로 구성되어 있다. 세 가지의 식이행동은 절제된 섭식, 정서적 섭식, 외부적 섭식으로 나뉜다. 각각의 식이행동에 해당하는 항목을 합산하여 세 개의 점수를 산출하고, 연속형 변수로 처리하여 분석했다. 세 점수와 SNP chip array data (Affymetrix Genome-Wide Human SNP Array 6.0)간에 연관성 분석을 실시하였다.

Results. 본 연구에서 가장 유의한 결과를 보인 single nucleotide polymorphism (SNP)은 Rs522723였으며, 정서적 섭식과 연관성을 보였다. 이 SNP은 다중비교의 문제를 해결하기 위한 FDR 보정 후에 유일하게 유의성을 보였다(0.04). 해당 변이가 포함되는 유전자는 CTDP1이며, 선천백내장, 안면형태이상, 신경병증후군(CCFDN)과 관련이 있는 것으로 알려져 있다. 그러나 위 변이 위치에서의 위험 대립형질의 수에 따라서 BMI로 측정된 비만 수준이 변화하지는 않는 것으로 나타났다.

Conclusions. 한국인에서 정서적 섭식 식이행동에 연관성 있는 특정 유전변이를 찾았다.

Keywords: 식이행동, DEBQ, CTDP1, 한국 가족-쌍둥이 코호트 연구, GWAS

Abstract

Genome-wide association study on eating behavior measured by Dutch Eating Behavior Questionnaire (DEBQ)

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Purpose. To investigate specific genetic variants which cause eating behavior problem in the Korean population.

Methods. Study subjects were 2606 healthy Korean adults who participated in the Healthy Twin study. Eating behavior problem were evaluated by Dutch Eating Behavior Questionnaire (DEBQ) which includes 33 items and diagnoses three different behaviors; restrained, emotional, and external eating behavior. We calculated three independent scores representing those three behaviors and treated them as continuous variables. Genome-wide association analysis has been done with eating behavior scores and SNP chip array data (Affymetrix Genome-Wide Human SNP Array 6.0).

Results. Rs522723 which associates with emotional eating was the most significant single nucleotide polymorphism (SNP) in our study. This SNP had a significant level of p-value(0.04) even after FDR correction. CTDP1 gene which include rs522723 is well-established to associate with congenital cataracts, facial dysmorphism and neuropathy syndrome (CCFDN). However, the number of risk allele at this locus does not seem to increase obesity level measured by BMI.

Conclusions. We searched for a genetic variant which play a role in emotional eating behavior in the Korean population.

Keywords: Eating behavior, DEBQ, GWAS, the Healthy Twin study

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I. Introduction

1. Background

Obesity is a rapidly growing public health challenge and it is the fifth leading risk for global death. WHO reported that 400 million obese adults in 2005 worldwide are obese and 700 million overlooked for 2015.[1] Obesity is a complex disorder caused by environmental and genetic factors both. Identifying the genetic cause of certain eating behavior may be clinically important in predicting risk of eating disorders and obesity-related complications. Eating behaviors, likewise, are common and complex psychiatric traits considered to be caused by both genetic and environmental factors.[2-5] They include a vast range of psychiatric phenomenon, specifically restraint, disinhibiting and hunger. Overeating driven by disordered eating behavior is one of many risk factors for obesity. The number of existing heritability study and GWAS of eating behavior are not abundant. However, several studies suggest that eating behavior and the individual domains of eating behavior have genetic influences. A study conducted in the Amish Family Diabetes Study confirmed that heritability of eating behavior is 23%-40%.[1] Also, Dutch Eating Behavior Questionnaire (DEBQ) score which will be described below in the Healthy Twin study Korea showed 21%-32% of genetic influence.[6] Yet, genome-wide association study of eating behavior has not been done. Twins and their family provide effective measures to evaluate genetic similarity and environmental sharing. Identical twins are genetically equivalent, and other relationships in a family such as siblings, parent-offspring and grandparent-grandchildren have different degree of genetic similarity and environmental sharing between the relationships is estimable by evaluating possible environmental factors. Analyzing

aforementioned information with genetic map, specific variant accounting for eating behavior is identifiable. Searching for a specific genetic variant which associates with eating behavior could be necessary to intervene obesity-related problems in regard of understanding psychological pathway.

1.1. Definition of eating behavior

Energy intake and energy expenditure, which are etiological causes of obesity, are consequences of behaviors such as choosing foods[7], eating foods, watching TV, playing sports. [8] These behaviors are influenced by a vast range of internal and external determinants. Therefore eating behavior does not automatically happen, but is processed by a variety of pressures, namely cultural, social, and psychological drives. To psychologically analyze motivation to eat which could lead to inadequate food consumption and overweight, theories have been developed. A research showed that differences in appetitive responsiveness are heritable.[4, 5] Therefore, weight gain seems to result from an interaction of genetically determined individual responsiveness to food and eating and the environment.[8]

1.2. Measurement of eating Behavior

There are some questionnaires to evaluate eating behavior; they generally investigate eating behavior with psychologically distinguished domains such as restraint, disinhibiting with emotional or external overeating, and hunger.[9, 10] The measure of eating behavior in this study is Dutch Eating Behavior Questionnaire (DEBQ) which is appropriate to evaluate eating behaviors based on psychological theories. The questionnaire is developed to improve

maintenance of treatment effects by fitting treatment and individuals[6]

Definition of Dutch Eating Behavior Questionnaire

Nisbett(1972) hypothesized that a person at normal weight and one in obese range both eat to reach the weight of 'set point' in the set point model and each person has different set point.[11] According to the set point model, obese person has higher set point that person at normal range of body weight. Thus, socially ideal weight standard make obese people to try to reduce their weight to lower than biologically "fit" level. These trials cause many different behavioral reactions including external reactivity in this model. Based on this model, Van Strien and et al. suggested that three categories of causes lead to dietary disorder. They invented Dutch Eating Behavior Questionnaire to demonstrate three basic theory; psychosomatic theory, externality theory and restraint theory.[12] Van Strien and et al. insisted that restrained, emotional, external eating should be distinguished properly. To demonstrate three theories which provide the basis for three eating behavior axes, they developed DEBQ. In psychosomatic theory, although normal reaction to emotionally arousal status such as anger, fear, and anxiety is reduction of appetite,[13, 14] some people react to these emotions with excessive eating.[15] In externality theory, regardless of internal status, external stimulus causes excessive intake.[16] At last, in restraint theory, a person who continuously restrains his or her own dietary intake less than the demand lose control for physical desire by disinhibitors periodically.[17, 18] 33 DEBQ-items measured with 5 point Likert scale includes 10 items each for restrained and external eating, 13 for emotional eating. The aim of this study is to identify genetic variants accounting for eating behavior measured by Dutch Eating Behavior Questionnaire (DEBQ) so as to predict severity of obesity by eating

behavior and reduce prevalence of obesity through health modification. DEBQ reliably and validly measures the three types of eating behavior measured.[10] the original version of DEBQ in English has been translated into different languages: Portuguese[9], Turkish[10], Spanish[11], French [12, 13], and Korean [14]. Validity and reliability, and also satisfactory internal consistency of these versions have been confirmed as good in a variety of ethnic group and age.

Other tools for evaluation of eating behavior

Most common measure of eating behavior is Three Factor Eating questionnaire (TFEQ), which was developed by Albert J. Stunkard and Samuel Messick in1985.[15] The TFEQ is usually applied in food intake-behavior related research and it consists of 51 items and measures three dimensions of eating behavior; cognitive restraint, disinhibition, and hunger, including 20, 16, and 15 items respectively. Each question scores either 0 or 1 point; therefore the minimum score is 0-0-0 and the maximum is 20-16-15. And there are revised questionnaires which have less number of items; TFEQ-R18 with 18 items[16, 17] and TFEQ-R21 with 21 items.[18]

CEBQ is a questionnaire to evaluate children's eating behavior. This questionnaire has been developed by Wardle and et al in 2001.[19] It asks children's parents to answer 35 items rated on a five-point likert scale that ranges from never to always. It contains eight scales of eating behavior: Food responsiveness, Emotional over-eating, Enjoyment of food, Desire to drink, Satiety responsiveness, Slowness in eating, Emotional under-eating, and Food fussiness. The instrument is ideal for use in research investigating the early precursors of eating disorders or obesity. It is validated to have a robust factor structure and good internal

reliability, but has not been validated against behavioral measures of eating.[20]

1.3. Eating behavior and obesity

Obesity level measured by body mass index (BMI) has been reported to relate to eating behaviors in several studies[11, 12, 21-23]. Also nature of food consumption[11, 21, 24-26] and psychological outcomes such as depression, anxiety or body-esteem[21, 27, 28] have been confirmed to link to those eating behaviors.

A study conducted in the same population as this study has reported that restrained eating and emotional eating were positively associated with weight gain and body mass index after adjusting other demographic factors and eating behaviors.[29] Also, increases in external eating score has shown to be significantly associated with weight gain regardless of age, education, weight, lifestyle at baseline, menopausal status at baseline(for women) and other eating behaviors of DEBQ in another research in the population.[30]

1.4. Genetic studies on eating behavior

There are studies searching for genetic evidence of eating behavior. A study which recruited 624 adults from 28 families participating in the Amish Family Diabetes Study has shown significant familial effects on eating behavior and suggestive genetic linkage by heritability analysis and a genome-wide multipoint linkage analysis.[31] Heritability estimates ranged from 0.23 +/- 0.09 for hunger, 0.28 +/- 0.09 for restraint and to 0.40 +/- 0.10 for disinhibition($P < 0.001$). In linkage analysis, 4 regions have come out to be suggestive: D3S1304 [LOD (log of odds) = 2.5, P = 0.0003] and D6S276 (LOD = 2.3, P = 0.0006) for restraint score, D7S657 [LOD= 1.6, P = 0.003] and D16S752 [LOD=1.4, P = 0.005] for

disinhibition, D3S1278[LOD=1.4, P = 0.005] for hunger.

Dutch Eating Behavior Questionnaire (DEBQ) score which will be described below in the Healthy Twin study Korea showed 21%-32% of genetic influence.[32] 2,144 Korean, adult, same-sex twins and their families was subject of this study. Heritability was estimated by variance component model and estimates were 0.31 +/- 0.036 for restraint, 0.25 +/-0.098 for emotional eating, 0.25+/-0.060 for external eating, respectively.

Specific genes associated with eating behavior and their biological functions have also been studied. Ghrelin is known to be associated with increased risk of binge eating[33] which is a pattern that a person rapidly consumes an excessive amount of food. Disinhibition which is also one of most frequently measured eating behaviors has been displayed association with genes TAS2R38 and GAD.[34, 35] FTO gene is a well-known to control satiety[36-38] and TAQ1A has shown an association with diminished reward response to food. [39-41]

2. Purpose

The aim of this study is to identify specific genetic variant accounting for abnormal eating behavior in Korean population. This study genetically mapped causal variants, not just calculate the overall genetic influences.

Sequentially, this study evaluate whether there is an increasing tendency of obesity level according to the number of risk alleles at locus identified in the previous step.

III. Method and study subject

1. Study subject

First, I explored 3320 Korean-population-based cohort which is twin and family-based individuals from the Healthy Twin study Korea. This is designated as a prospective and community-based cohort study in Korea with same-sex twins aged 30 or more. Specific design for this study has been addressed in a previous publication.[42] From whole study participants, our study involves 2606 adult individuals (1027 men and 1579 women) who have participated and fulfilled health examination related to obesity and Dutch Eating Behavior Questionnaire (DEBQ) in the Healthy Twin study Korea. Participants who agreed the informed consent answered 33 DEBQ items. Blood samples required for genome-wide analysis were also taken. Each items were summed up according to their behavior type so three scores were taken. Those scores were treated as continuous variables. The Healthy twin study Korea's protocol was approved by the ethics committees at the Samsung Medical Center and Busan Paik Hospital. DEBQ were, at first, self-completed and trained examiner checked validity of answers through interviews afterwards.

2. Method

2.1. Strategy of association analysis

Before scan genetic variants associated with three eating behavior types, conventional association models are concerned for covariate adjustment in further analyses. To investigate risk factors for eating behavior types, every possible combination of candidate risk factors are tested for association. All association are tested in a mixed model, which is a statistical model containing both fixed effects and random effects both. Mixed model in this study assumes that family members have high correlation in eating behavior scores so covariance structure for dependent variable is applied on each family. To compare fitness of tested models, Akaike Information Criterion (AIC) is used for model selection.

$$AIC = 2k - 2\ln(L)$$

where k : the number of parameters in the model

L : the maximized value of the likelihood function for the estimated model

Less AIC score means better fitness of models. A model with only significant independent variables and less AIC score will be chosen as the fittest model for each eating behavior. Association test in mixed model and AIC score calculation are performed in SAS 9.3..

2.2. Genotyping

Genomic DNA was extracted out of blood samples drawn from all participants at their first recruitment in 2005. Extracted DNA was genotyped in the study centers with the Affymetrix Genome-Wide Human SNP Array 6.0. Carried out quality control (QC)

procedures are following and SNPs unmet this norm were excluded: duplicated SNPs (3,011); Hardy – Weinberg Equilibrium (HWE) <.001; Minor Allele Frequency (MAF) <.01; genotype missing rate >.05 (292,653); Mendelian error >3 families (11,456); and non-mendelian error > 3 families (47,594). These exclusions reduced the total number of markers from 871,166 to 516,452.

2.3. Statistical analysis

i. Heritability analysis

Genetic heritability can have two meanings in its sense. The narrow-sense of heritability is the relative quantity of additive genetic components over total trait variance. Next, broad-sense of heritability is more comprehensive concept than genetic heritability, as containing effects of familial environment as well as additive genetic factors.

Heritability has been statistically defined by two approaches; Morton's correlation and regression methods using path analysis[42] and Fisher's variance decomposition concept[43]. These two approaches are based on polygenic model which assumes that a phenotype is determined of many genes, each with small, linear and additive effects.

Basic polygenic model suppose that phenotype (P) is a function of genetic (G) and environmental effects.

$$P= G+E$$

Therefore, total phenotypic variance is explained by genetic and environmental variance.

$$V_G = V_G+ V_E$$

The broad-sense genetic heritability, also called as multifactorial heritability, is defined as relative quantity of the total phenotype variance to genetic variance as genetic effect.

$$h^2_B = V_G/V_P$$

There is another model which divide this genetic variance into additive effects and dominance deviations.

$$V_G = V_A + V_D$$

Then, as I mentioned above, this decomposition make us possible to estimate the narrow-sense genetic heritability, defined as relative magnitude over additive genetic effects.

$$h^2_N = V_A / V_P$$

Moreover, we should consider the environmental effect in this genetic model. The environmental effect can be decomposed into common familial (C) and random non-familial (R) environmental effects.

$$V_P = V_A + V_D + V_C + V_R$$

The C factor, familial environmental variance component which means the cultural heritability, could be defined as relative variance of additive familial environmental effects to the total phenotypic variance.

$$C^2 = V_C / V_P$$

Different models underlying the heritability estimation may lead us to interpret results differently. The models could assume linearity and additivity, assortative mating, and

the underlying distribution of the data.

The narrow sense of heritability has been estimated in this study. The proportion of additive genetic effect over total phenotypic variance is defined as heritability.

$$h^2_N = V_A / V_P$$

Additionally, the familial environmental factor, also called as the C factor, is defined as below.

$$C^2 = V_C / V_P$$

Two models are tried in this analysis; AE and ACE model. AE model assumes that there is only additive genetic factor to explain total phenotypic variance and the rest of explained by the A factor is the random non-familial environmental factor. In ACE model, the C factor, familial environmental effect is estimated additionally. So the whole environmental effect is divided into the familial one and the non-familial one.

All analyses are performed on an assumption of normal multivariate distribution. Therefore, traits which do not follow normal distribution are necessary to be transformed to follow it. SOLAR software, which I used for the heritability analyses, has an option named ‘inormal’. This option support normalization of phenotype scores so that we could get proper result for analyses.

ii. Genome-wide association analysis

For the family-based association test, we used a generalized transmission disequilibrium test19, 20, calculates statistics (U statistics), which is a covariate between a trait and the genotype score of a subject where the genotype score is the deviation from the expected number of risk alleles estimated by the gene pool of the family21. For the calculations, only parental genotype information, but not their phenotype information, is used. Thus, the unused association information between genetic markers and phenotype of founders are additionally extracted using test methods for unrelated individuals. The general "FBAT"20 statistic U is based on a linear combination of offspring genotypes and traits. The test statistic uses a natural measure of association between two variables, a covariance between the traits and the genotypes. We define the covariance as

$$U = \sum T_{ij}(X_{ij} - E(X_{ij}|S_i))$$

Where i: family

j: non-founders in the family

T_{ij}:a centered phenotype

(X_ij-E(X_ij | S_i)):residual of 'transmission' of parental genotype to offspring

in which Xij denotes some function of the genotype of the j-th offspring in family i at the locus being tested. The Tij is the coded trait, depending upon possibly unknown parameters. In general, the coding for Tij is specified as Yij - µij. Here, Yij denotes the observed trait of the j-th offspring in family i, and µij is seen as an offset value.[43, 44]

For the population-based approach, PLINK 22 regression models were used to test for an association between the 516,452 markers and the residual phenotype. Parental correlations with their children were not considered in these analyses. The analyses were done with the PLINK program package, Version 1.07.[45] Plink performs linear- and logistic-regression models for quantitative-trait association.[45] It can also include multiple binary or continuous covariates having both main effects and interactions. In this stud age, sex which have been associated every eating behaviors are included as covariates.

Several studies have discovered that family-based association can be strengthened by parental association test result and independent information from both sets can be combined.[45, 46] Won has shown that information about the effect sizes can be used to obtain the best weights for Liptak's method of combining p-values.[47] In this study, I combined independent association result derived from both family-based and unrelated population-based methods by Won's method.[47] Won's method performs an optimally weighted z-test and the combined p-value is defined as below.

$$p_Z = 1 - \Phi\left(\frac{\sum_{i=1}^{k} w_i Z_i}{\sqrt{\sum_{i=1}^{k} w_i^2}}\right)$$

where $Zi = \Phi - 1(1 - pi)$,
pi : a P -value for the i-th study of k studies in total
wi: are weights
Φ, Φ^{-1} : the standard normal cumulative distribution function and its inverse.

P-values resulted from association tests would have a multiple comparison problem. Because more than one hypothesis is tested simultaneously, probability of rejecting the null hypothesis should be lower than when just one hypothesis is tested. To solve this problem, we adapted False Discovery Rate for adjustment. In the below formula, p-values are first sorted in a decreasing manner, and then they are adjusted by a probability that false positive cases happens over whole rejection of null hypothesis. .

$$FDR = Q_e = \mathrm{E}[Q] = \mathrm{E}\left[\frac{V}{V+S}\right] = \mathrm{E}\left[\frac{V}{R}\right] \text{ Where } \frac{V}{R} \text{ is defined to be 0 when } R = 0.$$

V : the number of false positives (Type I error) (also called "false discoveries")

S : the number of true positives

T : the number of false negatives (Type II error)

U : the number of true negatives

R : the number of rejected null hypotheses (also called "discoveries")

Q : the proportion of false discoveries among the discoveries

Display of result of genome-wide association scan

i. Manhattan plot

A Manhattan plot is a scatter plot, used to display data with a large number of data-points, for example in genome-wide association studies (GWAS).[48] In GWAS Manhattan plots, genomic coordinates are placed along the X-axis and negative logarithm of the association P-value for each single nucleotide polymorphism are displayed on the Y-axis, displaying stronger association with smaller p-value locates higher position on the Y-axis. “The Manhattan plot” is named after the view from sky in Manhattan, having skyscrapers above the lower level buildings.

ii. Regional plot

Due to the enormous number of single nucleotide polymorphism, the plot cannot show hotspots, which have the highest negative logarithm of p-values, in a clearer manner. The regional plot zooms in the region which has a peak among the whole SNPs to better observe significant result.

IV. Results

1. Epidemiology of eating behavior

1.1. Distribution of eating behavior

In table 1, characteristics of study subjects are described. Among 2606 study subjects, 60.59% was female and average age was 44. The age of study population ranges from 17 to 81. Thirty's is most plentiful (39.79% of total population) and age distribution does not follow normal distribution, tested by Kolmogorov-Smirnov fit test.

Mean of restrained eating score, emotional eating score and external eating score are 2.34±0.99, 1.44±0.67, 2.67±0.77, respectively. Distributions of restrained eating and emotional eating score are skewed to right (Figure 1 and 2), although that of external eating seems to follow the normal distribution.(Figure 3)

To assume normal multivariate distribution for genetic analyses, I applied normalization methods for restrained and emotional eating scores. I described more specific methodologies in III. Method.

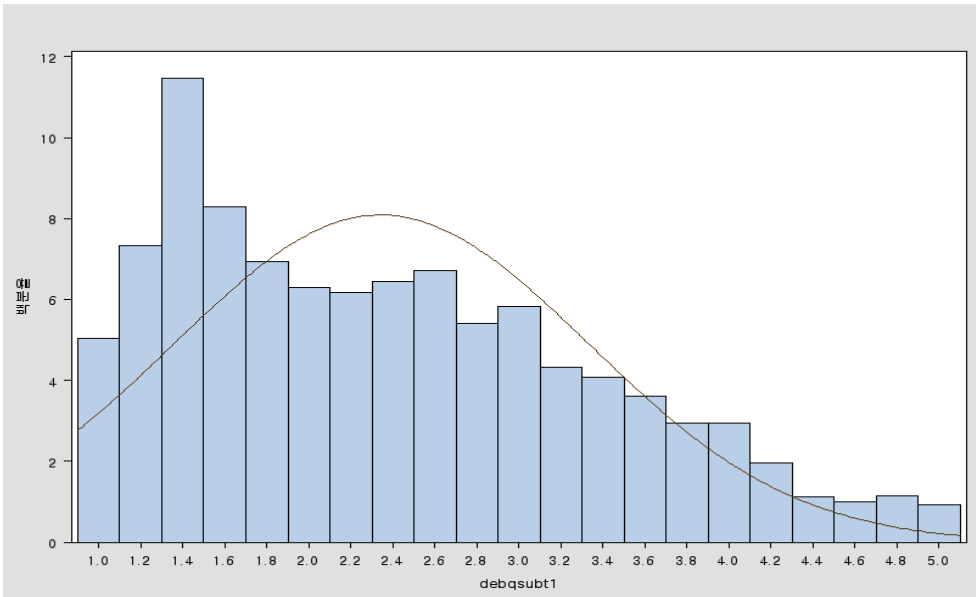

Figure 1 Distribution of Restrained Eating Score

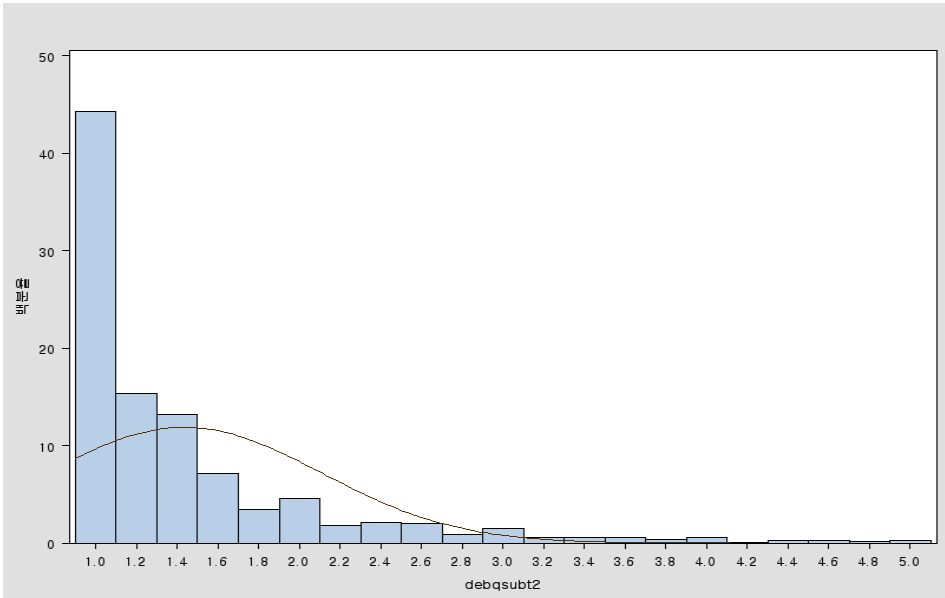

Figure 2 Distribution of Emotional Eating Score

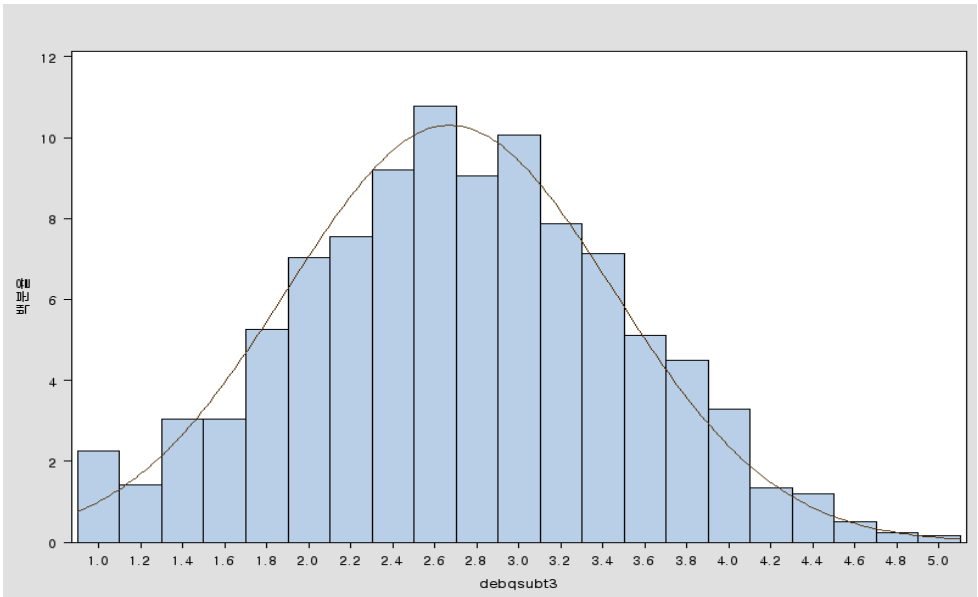

Figure 3 Distribution of External Eating Score

In table 1, I described summary statistics of three eating behaviors and main risk factors for those eating behaviors. We performed t-test to test the null hypothesis; H_A: No differences between female and male exist. Female tends to have higher score in every behavior and the differences are statistically significant. Also, sex differences in physical activity, calorie intake, BMI and weight were significant enough to reject the null hypothesis. Sex can be confounder in a search of association; therefore it needs to be adjusted for further association analysis.

According to the age distribution, I divided our subjects into 4 different age groups; 17 to 29, 30 to 39, 40 to64 and more than 65 so than each groups have similar number of population. Among four age groups, I tested the null hypothesis; H_0: eating behavior scores in every age group are same by ANOVA. In every eating behaviors, the scores gets lower as group's age gets older. Restrained eating score represented a significant difference in different age groups. According to the Tukey's grouping, more-than-65 age group had significantly different restrained eating score. Emotional eating and external eating scores were also significantly different in four age groups. In regard of risk factors for eating behaviors, except for weight, BMI, physical activity and calorie intake showed significant differences in four age groups. For further analyses; phenotypic association analysis, heritability analysis and genome-wide association analysis, we hired different strategies according to the purposes. In phenotypic association study, I would like to look at the association between eating behaviors and possible risk factors. However, in heritability analysis and genome-wide association analysis, I would like to see genetic influence or association including influences of BMI, energy consumption and expenditure, rather than getting rid of the etiological effects.

In Table 2, I showed mean±S.D. for three eating behavior types according to sex and age group. External eating score is the highest in all age groups regardless of sex. Restrained eating score was little lower than that and score for emotional eating is far lower than other scores in all categories. I performed t-test for sex difference in eating behavior score for all age groups. Individuals aged more than 65 had no significant differences between male and female in all eating behaviors. For restrained eating behavior, there were significant differences in all age groups; female tends to have higher score than male. The youngest age group has the biggest differences in emotional eating score, and the degree of difference was similar in other age groups. Additionally, differences for external eating scores in all age groups except for who aged more than 65 are in similar magnitude.

Table 2 Characteristics of study population Mean± **S**. **D**

	Eating behavior N=2606			**Physical activity N=2325**	**Calorie intake (Kcal) N=2606**	**BMI ($kg/m^{2)}$) N=2606**	**Weight (kg) N=2606**
	Restrained eating	Emotional eating	External eating				
Sex							
Female(n=1579)	2.55±1.00	1.52±0.74	2.76±0.78	5720.19±9512.60	1764.22±856.73	23.15±3.30	56.92±8.30
Male(n=1027)	2.03±0.88	1.30±0.52	2.54±0.75	7056.19±10848.84	1956.24±813.29	24.41±3.05	70.18±10.48
Difference test	0.52*	0.23*	0.21*	-1336.0*	192.0*	-1.26*	13.27*
Age group							
17≤age<30(n=194)	2.41±0.96	1.66±0.78	3.02±0.68	4376.06±7423.99	1972.39±776.82	22.35±3.74	62.05±15.05
30≤age<40(n=1037)	2.38±0.92	1.49±0.69	2.86±0.70	4865.86±8148.81	1931.14±812.05	23.07±3.17	61.84±11.76
40≤age<65(n=1128)	2.35±1.04	1.37±0.63	2.54±0.78	7817.49±11564.23	1794.25±876.52	24.26±3.13	62.60±10.32
65≤age(n=247)	2.10±0.96	1.31±0.57	2.23±0.77	6573.00±11149.21	1561.23±808.34	24.33±3.09	61.42±9.72
ANOVA	0.0006	<.0001	<.0001	<.0001	<.0001	<.0001	0.3055
Total	2.34±0.99	1.44±0.67	2.67±0.77	6237.35±10069.71	1839.90±844.95	23.65±3.26	62.14±11.27

Table 3 Eating Behavior Score Mean by sex and age groups

Mean± S.D

Age group	Variable	Sex		Difference
		Male	Female	
17≤age<30(n=79)	Restrained eating	2.12±0.88	2.61±0.97	-0.50*
	Emotional eating	1.41±0.57	1.83±0.86	-0.42*
	External eating	2.89±0.68	3.10±0.67	-0.21*
30≤age<40(n=406)	Restrained eating	2.08±0.84	2.58±0.92	-0.50*
	Emotional eating	1.34±0.56	1.59±0.75	-0.25*
	External eating	2.73±0.68	2.95±0.70	-0.22*
40≤age<65(n=415)	Restrained eating	1.93±0.87	2.59±1.06	-0.66*
	Emotional eating	1.23±0.46	1.45±0.71	-0.22*
	External eating	2.37±0.75	2.63±0.78	-0.27*
65≤age(n=127)	Restrained eating	2.12±0.99	2.08±0.92	0.05
	Emotional eating	1.31±0.50	1.32±0.64	-0.001
	External eating	2.30±0.78	2.16±0.75	0.15

1.2. Internal correlation of Dutch Eating Behavior Questionnaire(DEBQ) subscales

Three subscales of eating behavior showed low level of correlation, especially external eating has moderate level of correlation, the coefficient is 0.45.(Table 4) However, in our study, no analysis involving eating behaviors as independent variable will be performed.

Table 4 Correlation structure of eating behavior subscales

Each cells has correlation coefficient and the probability of rejecting the null hypothesis P<|r|.

Correlation coefficients			
	Restrained Eating	Emotional eating	External eating
Restrained eating	1.00		
Emotional eating	0.28 <.0001	1.00	
External eating	0.24 <.0001	0.45 <.0001	1.00

1.3. Risk factors for abnormal eating behaviors

I performed association test for restrained eating behavior, among suspected risk factors; BMI, weight, calorie intake as energy consumption and physical activity as energy expenditure. All the combinations of four main risk factors were tried to be involved in association models. The number of possible combinations is fifteen. I expected there is genetic correlations among family members, therefore performed mixed model based association test. Additionally, I hired 4 different criteria to compare fitness of models.

1.3.1 Risk factors for restrained eating

Using -2 Res Log Likelihood, AIC, AICC and BIC model selection criterion, I decided the fittest model for restrained eating in this analysis. Smaller number for every criterion shows better fitness. Age and sex showed high significance for all possible models, so I displayed models only including age and sex. Physical activity did not show significance in association models so models with physical activity are excluded from candidates. Therefore, the fittest model with smaller criterion scores and significant independent variables for restrained eating is that of age, sex and BMI. In Genome-wide association test which would be main purpose of this study will be performed after adjusted age and sex effect. Influence of BMI will not be excluded to see if there is any genetic variant representing association between BMI and eating behaviors.

Table 5 Independent variables for restrained eating

Model selection criteria	Included variables (not specifying age and sex)														
	Sex, Age, BMI	Sex, Age, Weight	Sex, Age, Calorie intake(CI)	Sex, Age, Physical activity (PA)	Sex, Age, BMI, Weight	Sex, Age, BMI, CI	Sex, Age, BMI, PA	Sex, Age, Weight ,CI	Sex, Age, Weight, PA	Sex, Age, CI, PA	Sex, Age, BMI, Weight , CI	Sex, Age, BMI, Weight , PA	Sex, Age, Weight , CI, PA	Sex, Age, BMI, CI, PA	Sex, Age, BMI, Weight, CI, PA
AIC	6952.7	6969.9	7081.3	6368.6	6960.2	6957.8	6256.1	6975.0	6272.5	6383.6	6965.0	6263.3	6281.1	6264.9	6271.7
Significan -ce For variables in each model	<.0001 <.0001 <.0001	<.0001 <.0001 <.0001	<.0001 <.0001 0.0059	0.0006 <.0001 0.1653	<.0001 <.0001 <.0001 0.2221	<.0001 <.0001 <.0001 0.0001	<.0001 <.0001 <.0001 0.0865	<.0001 <.0001 <.0001 0.0001	0.0006 <0.0001 <0.0001 0.1455	0.0002 0.0001 0.0353 0.2417	<.0001 <.0001 <.0001 0.1742 0.0001	<.0001 <.0001 <.0001 0.1923 0.0970	0.0001 <.0001 <.0001 0.0010 0.2647	<.0001 <.0001 <.0001 0.0011 0.1674	<.0001 <.0001 <.0001 0.1459 0.0009 0.1888

1.3.2 Risk factors for emotional eating

I followed the same methodology to select the fittest model as association test of restrained eating. According to four model selection criterion, I also decided the fittest model for emotional eating. The model with the smallest number in each criterion and risk factors which previously showed statistical significance in their univariate association tests. Again, physical activity does not show significance in its univariate association test so models with weight are excluded from candidates. Therefore, the fittest model with smaller number and significance of all included variables is that of age, sex, BMI and calorie intake. For further genetic test, we will exclude BMI and calorie intake from confounding variables to see effect involving obesity-related association.

Table 6 Independent variables for emotional eating

Model selection criteria	Variables included														
	Sex, Age, BMI	Sex, Age, Weight	Sex, Age, Calorie intake(CI)	Sex, Age, Physical activity (PA)	Sex, Age, BMI, Weight	Sex, Age, BMI, CI	Sex, Age, BMI, PA	Sex, Age, Weight, CI	Sex, Age, Weight, PA	Sex, Age, CI, PA	Sex, Age, BMI, Weight, CI	Sex, Age, BMI, Weight, PA	Sex, Age, Weight, CI, PA	Sex, Age, BMI, CI, PA	Sex, Age, BMI, Weight, CI, PA
AIC	5149.3	5151.0	5171.0	4714.3	5156.0	5138.7	4674.8	5140.9	4675.2	4692.7	5146.0	4680.5	4662.1	4660.9	4667.5
Significance for variables in a model	<.0001 <.0001 <.0001	<.0001 <.0001 <.0001	<.0001 <.0001 <.0001	<.0001 <.0001 0.8693	<.0001 <.0001 0.1048 0.0781	<.0001 <.0001 <.0001 <.0001	<.0001 <.0001 <.0001 0.7406	<.0001 <.0001 <.0001 <.0001	<.0001 <.0001 <.0001 0.8453	<.0001 <.0001 <.0001 0.4068	<.0001 <.0001 0.1137 0.1167 <.0001	<.0001 <.0001 0.1382 0.0457 0.7951	<.0001 <.0001 <.0001 <.0001 0.4289	<.0001 <.0001 <.0001 <.0001 0.3561	<.0001 <.0001 0.1456 0.0809 <.0001 0.3939

1.3.3 Risk factors for external eating

In this association analysis of external eating, according to the same steps were hired. The fittest model has been determined by four model selection criterion scores. The fittest model to explain external eating score was that of age, sex, weight and total calorie intake. However, for the genetic analysis which will be performed later in this study(section Ⅳ.2 and Ⅳ.3), I will adjust for age and sex only to see genetic association or influence including obesity-related effect.

Table 7 Independent variables for external eating

Model selection criteria	Variables included														
	Sex, Age, BMI	Sex, Age, Weight	Sex, Age, Calorie intake(CI)	Sex, Age, Physical activity (PA)	Sex, Age, BMI, Weight	Sex, Age, BMI, CI	Sex, Age, BMI, PA	Sex, Age, Weight ,CI	Sex, Age, Weight , PA	Sex, Age, CI, PA	Sex, Age, BMI, Weight , CI	Sex, Age, BMI, Weight , PA	Sex, Age, Weight , CI, PA	Sex, Age, BMI, CI, PA	Sex, Age, BMI, Weight , CI, PA
AIC	5679.6	5672.5	5652.1	5007.7	5674.9	5657.1	5009.5	5651.8	5002.8	4986.1	5653.8	5006.1	4986.0	4990.8	4989.0
Significan -ce for variables in a model	<.0001 <.0001 0.0107	<.0001 <.0001 <.0001	<.0001 <.0001 <.0001	<.0001 <.0001 0.9866	<.0001 <.0001 0.0261 0.0002	<.0001 <.0001 0.0483 <.0001	<.0001 <.0001 0.0081 0.9871	<.0001 <.0001 0.0007 <.0001	<.0001 <.0001 <.0001 0.9761	<.0001 <.0001 <.0001 0.5344	<.0001 <.0001 0.0211 0.0003 <.0001	<.0001 <.0001 0.0473 0.0003 0.9196	<.0001 <.0001 0.0009 <.0001 0.5657	<.0001 <.0001 0.0440 <.0001 0.5314	<.0001 <.0001 0.0393 0.0008 <.0001 0.6153

2. Heritability analysis

Genetic influences estimated over each eating behavior were 33%(±3%) for restrained eating, 39%(±3%) for emotional eating and 40%(±3%) for external eating. Heritability of eating behavior spans from 33 to 40 percent. In obesity-related traits, genetic influence was considerably stronger spanning from 54 to 65 percent. (Table 4)

Different ACE models group participants differently according to types of environmental factors in demand. The household effect represents the environmental effect shared by family members who live together; thus sharing most lifestyles. The common environmental effect for restrained eating was not significant. And that for emotional eating behavior and external eating behavior was 14% and 12%, respectively. Thus, more than 10% of phenotypic variance in emotional and external eating behavior can be explained by common environmental influence which is shared between family members.

Table 8 Heritability estimates of eating behavior and obesity-related traits

	AE model	ACE models!
		Household effect
Eating behavior types		
Restrained eating	0.33±0.03	0.30±0.05 0.03±0.03$^{N.S.}$
Emotional eating	0.39±0.03	0.18±0.06 0.14±0.03
External eating	0.40±0.03	0.24±0.05 0.12±0.03
Obesity-related traits		
Body Mass Index	0.65±0.03	0.65±0.03
Waist-Circumference	0.54±0.03	0.54±0.03

*estimates±S.E.

!Each cells in ACE model shows additive genetic heritability above and common environmental influence below.

3. Genome-wide association study

In this GWA analysis of three eating behaviors; namely restrained, emotional, and external eating, we detected association of genetic variants with P<10-5 as significant. In the table 5, we listed top significant SNPs associated with each eating behaviors. In Figure 4, we displayed all SNPs' location with their significance.[49] QQ plots of association of three eating behaviors are in supplementary figure 1.

To avoid multiple comparison problems, we adjusted p-values with False Discovery Method. In table 5, after the adjustment, there was only one SNP which is significant with less than 0.05. The SNP is rs522723 associated with emotional eating behavior and we observed that there are several SNPs showing a peak around rs522723, even though the other did not have genome-wide significances.

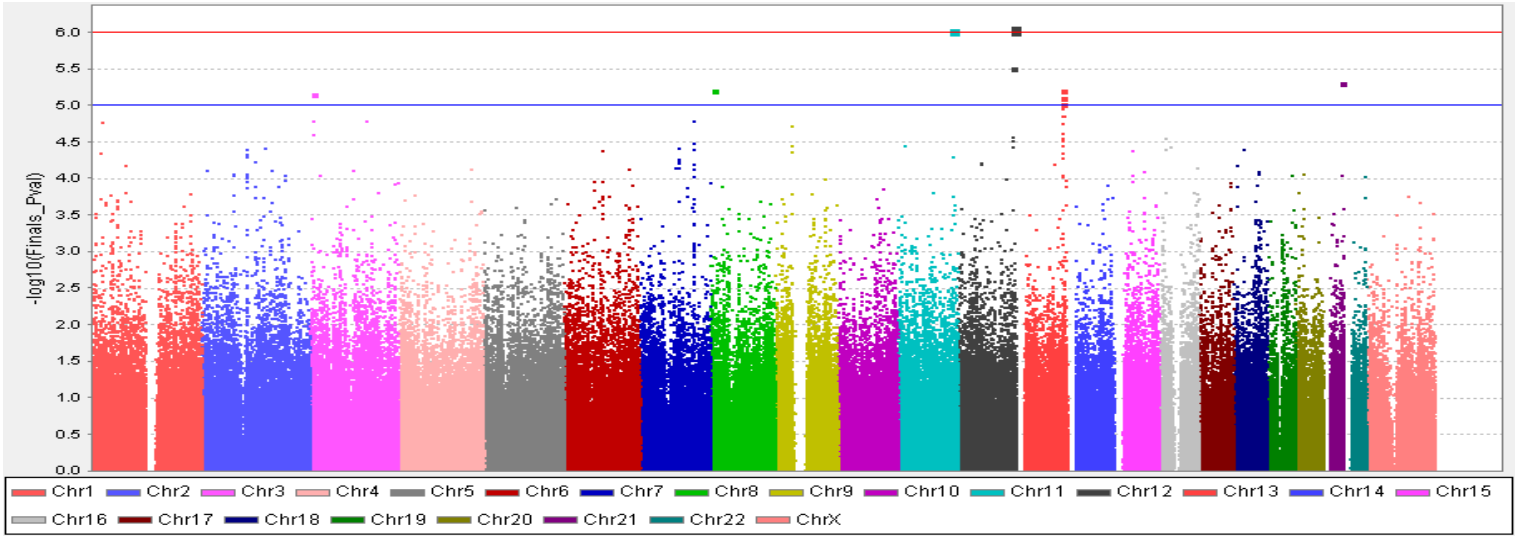

Figure 4 SNPs associated with restrained eating

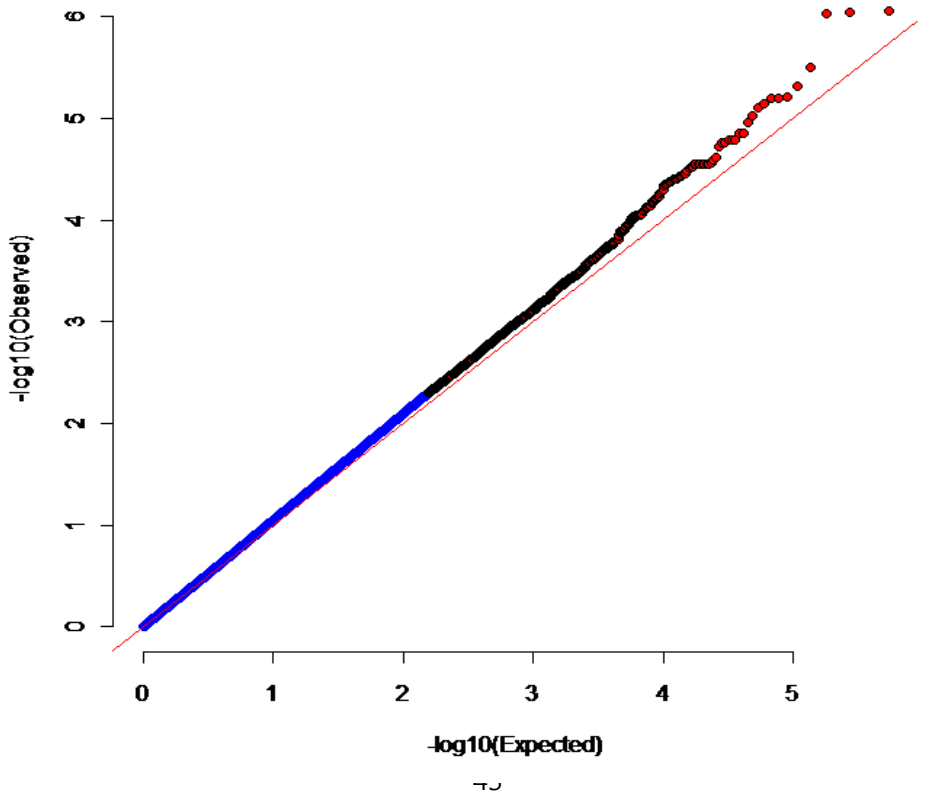

Figure 5 QQ Plot of Restrained Eating Score GWAS Result

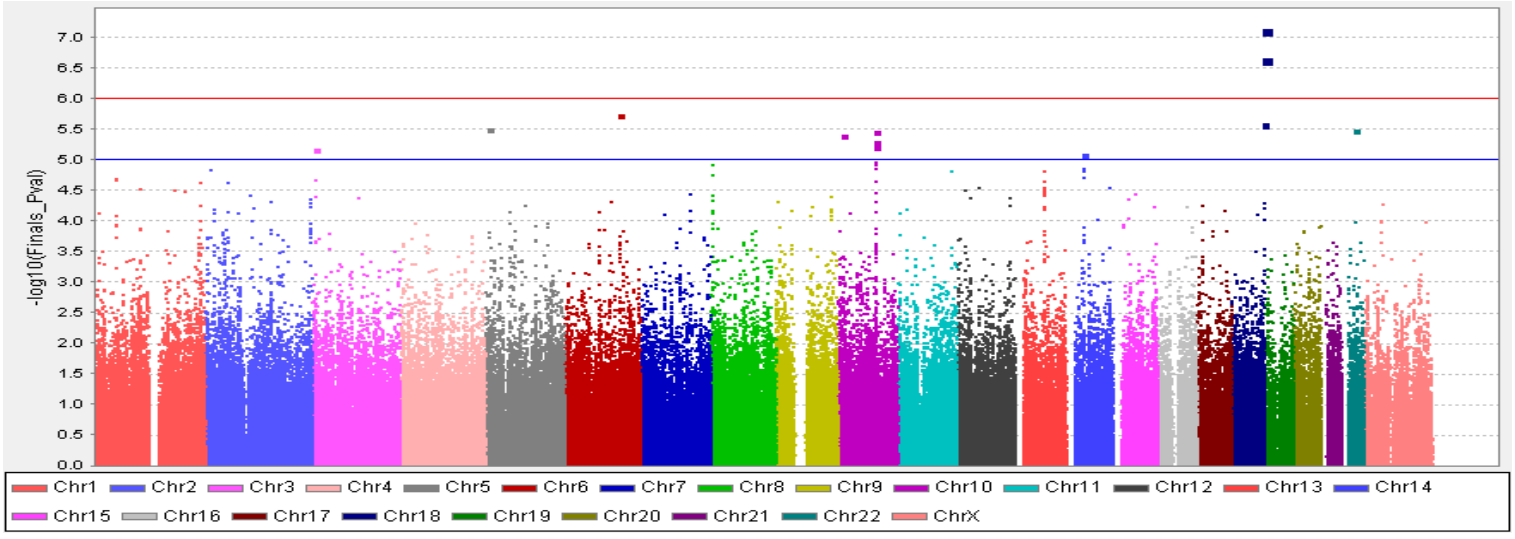

Figure 6 SNPs associated with emotional eating

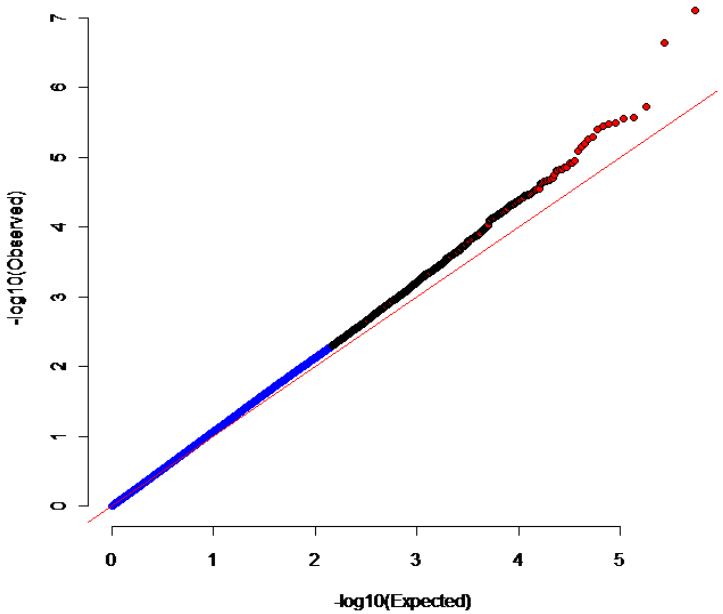

Figure 7 QQ Plot of Emotional Eating Score GWAS Result

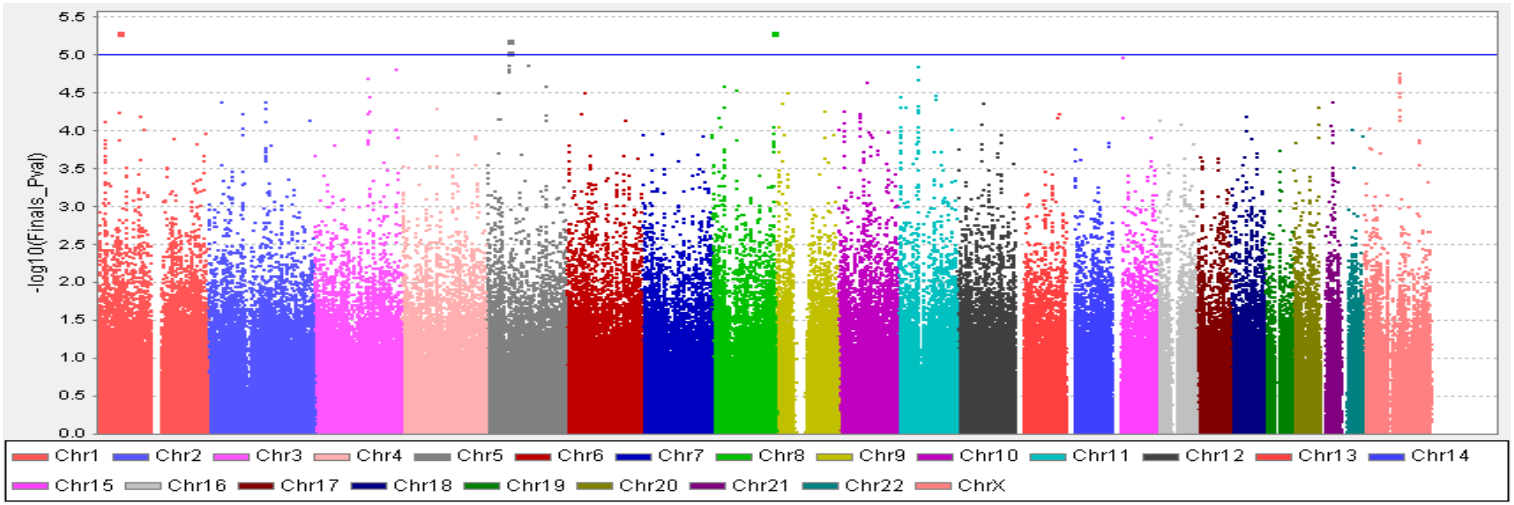

Figure 8 SNPs associated with external eating

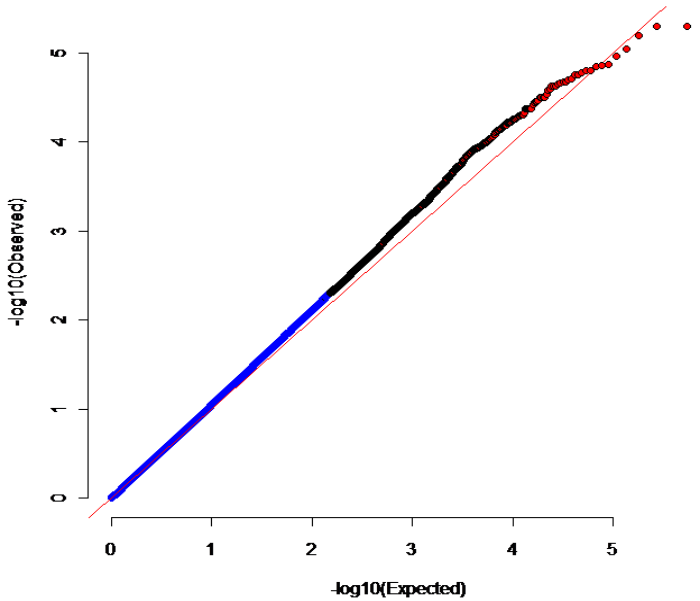

Figure 9 QQ Plot of External Eating Score GWAS Result

Table 9 A list of single nucleotide polymorphisms associated with three eating behavior types

SNP	Chr	position	Minor Allele	Unrelated association p-value	Family-based association p-value	Combined P-value	P-value after adjustment	Associated gene
Restrained eating								
rs1969207	12	125745397	G	8.5E-4	2.59 E-04	8.65E-07	0.165009	LOC100996671
rs9645663	11	118379050	C	2.49 E-04	1.02 E-03	9.12E-07	0.165009	CCDC84
rs10847204	12	125745113	T	8.92 E-04	2.62 E-04	9.23E-07	0.165009	LOC100996671
rs1355555	12	127186202	A	1.85 E-04	4.95 E-03	3.10E-06	0.415304	LOC100996671
rs8131547	21	44320989	T	1.13 E-05	4.28 E-02	4.88E-06	0.415304	NDUFV3
Emotional eating								
rs522723	18	75574510	T	1.98 E-05	5.31 E-04	7.68E-08	0.041163*	CTDP1
rs4799084	18	75634606	T	1.13 E-04	2.51 E-04	2.25E-07	0.060382	CTDP1
rs9320884	6	122937612	C	7.45 E-04	5.37 E-04	1.86E-06	0.238002	PKIB
rs12605631	18	77450915	C	6.58 E-04	8.73 E-04	2.66E-06	0.238002	CTDP1
rs2510276	18	77484550	C	2.38 E-04	1.93 E-03	2.80E-06	0.238002	CTDP1
External eating								
rs1158942	1	46911921	C	1.55 E-03	9.01 E-04	4.98E-06	0.491026	LOC729041
rs4736080	8	140579392	T	1.82 E-04	7.11 E-03	5.08E-06	0.491026	KCNK9
rs2456199	5	52182162	T	3.20 E-02	1.5 E-05	6.35E-06	0.491026	ITGA1
rs2456223	5	52177346	A	2.32 E-02	4.6 E-05	9.09E-06	0.491026	ITGA1

*statistically significant after adjustment for multiple comparison error correction

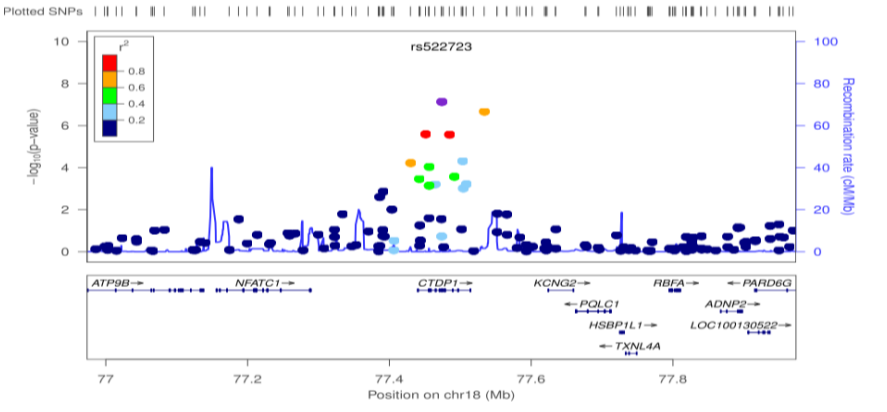

Figure 10 Regional plot of rs522723

Point colored purple is the rs522723, which is the most significant SNP in this study. There are relatively significant SNPs around rs522723 having a peak in CTDP1 gene.

In Figure 3, we zoomed in a smaller region around rs522723 using LocusZoom, the web-based software.[50] We clearly see that rs522723 and other SNPs in CTDP1 gene have relatively significant signals. This gene encodes a protein which interacts with the carboxy-terminus of the RAP74 subunit of transcription initiation factor TFIIF, and functions as a phosphatase that processively dephosphorylates the C-terminus of POLR2A (a subunit of RNA polymerase II), making it available for initiation of gene expression. Mutations in this gene are associated with congenital cataracts, facial dysmorphism and neuropathy syndrome (CCFDN). Alternatively spliced transcript variants encoding different isoforms have been described for this gene. [provided by RefSeq, Feb 2011][51-55]

4. Contribution of eating behaviors to obesity

Association between obesity measured by body mass index (Chatalet's index) and eating behavior subscales.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	61.205324	61.205324	64.45	<.0001
Error	2604	2472.961019	0.949678		
Corrected Total	2605	2534.166343			

Table 10 Association analysis result of eating behavior subscales

Eating behavior subscales	F value	Pr > F	R square
Restrained eating	64.45	<.0001	0.024152
Emotional eating	12.45	0.0004	0.004759
External eating	7.31	0.0069	0.002798

Three subscales of eating behavior have shown significant association with BMI. However, the proportion of BMI data which eating behavior scores explain is only less than 2 percent.

ANOVA of obesity levels by the number of minor alleles

We hypothesized that obesity level measured by BMI would increase by the number of risk alleles in eating behavior. However, obesity levels did not represent significant differences according to the number of risk alleles. ANOVA results in 0.1734 of p-value and the number of risk alleles accounts only for 0.5 percentage of our data. This may mean the most significant variant in our study does not have a genetic influence to obesity or there are more complex pathway from this variant to be expressed in a problematic obesity level.

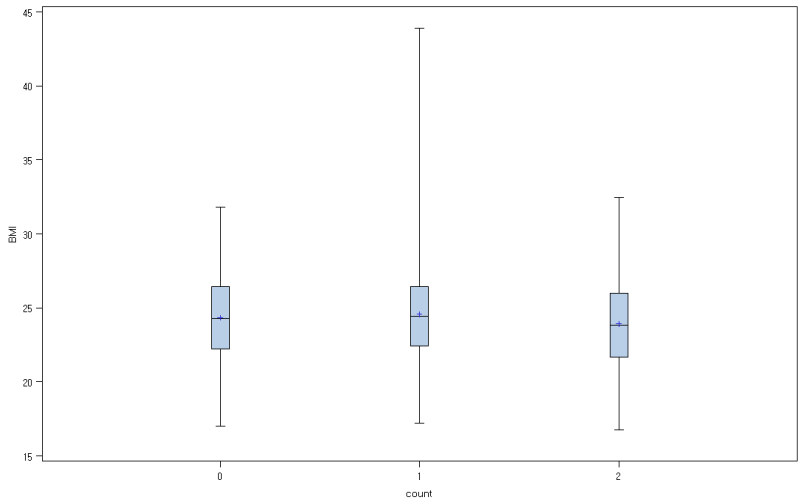

Figure 11 Boxplot of BMI levels by the number of risk alleles

V. Discussion

1. Discussions

Study participants showed difference in eating behavior scores according to their gender. Female tends to have higher scores. This phenomenon supports that eating behavior is influenced by environmental risk factor. Therefore to see the genetic association between specific variant and eating behavior score, sex should have been controlled. With respect to age effect, there is no trend according to age increase, however all age groups did not show similar score, but the oldest age group, more than 65, tended to have higher scores. This result also represent that there could be environmental effect in eating behavior, so I adjusted age effect in heritability and genome-wide association analysis.

Emotional eating behavior means abnormal reaction to emotionally arousal status. [56, 57] Rs522723 has come out as the most significant SNP in our study and it associates with emotional eating problem. CTDP1 gene which includes rs522723 relates to TFIIF and RNA polymerase II that play essential role in every part of human body. It is too vast to specify its function in a eating behavior; however there could be subsequent study to explore the pathway from CTDP1 or rs522723 to eating behavior trait.

There is no sufficient evidence on the genetic architecture of eating behaviors. But they are known to have genetic and environmental influences both. Usually, common genetic risk variants have relatively low penetrance and are responsible for a small

increase in disease risk.[58, 59] This could explain the discrepancy in our results that rs522723 did not play a role as a risk factor for obesity. Obesity is a complex trait and it is well-known to result from genetic and environmental influences.[60] Except for eating behavior, a plethora of factors causes obesity in that there are several pathways to obesity. Therefore we could not place weight on eating behavior as a main cause of this disease and the discrepancy reflects this issue.

In obesity studies, there are rare monogenic evidences. Mutation on Leptin gene or its receptor (LEPR) showed abnormal eating behavior and individuals with the mutations had childhood morbid obesity.[61, 62] Also, individuals with leptin deficiency experienced satiety improvement and weight loss after Leptin replacement.[63] α-MSH, α-melanocyte stimulating hormone, synthesis which promotes satiety is enhanced by Leptin.[64] This kind of discoveries could lead to pathways controlling eating behavior and energy metabolism system. However, discovered monogenic variants associated with obesity explains only less than 10 percent of obesity epidemic.[65] Therefore the genome-wide association study result in this dissertation, rs522723 has accounted for obesity as little as less than two percent may be explained by complex pathway of obesity and energy homeostasis.

In association studies, the feasibility of finding causal variant underlying complex traits depends upon following factors; the ability to measure the trait reproducibly on a large number of subjects, demonstration that the trait is significantly heritable and is likely influenced by genetic factors, and an appropriate study design and analysis methodology that will yield sufficient power to detect loci with plausible effect sizes.[41]

In regard of these factors, this study has checked validity of result through QQ plot, and design of underlying cohort study has already been proved to be appropriate. Also, eating behavior has been shown genetic influences in different population, influenced by both genetic and environmental factors. However, result of this study represents only one significant SNP. This disappointing result could be due to small effect size of risk allele and low level of phenotypic variation. Study population in this study is healthy adult; they had low eating behavior scores for which statistical test could hardly search.

In heritability study conducted on the same population with this study has shown consistent results in high heritability. In the existing study, heritability of eating behaviors was 0.31 +/- 0.036 for restraint, 0.25 +/-0.098 for emotional eating, 0.25+/- 0.060 for external eating.[32] The heritability of eating behavior in this study was 33%(±3%) for restrained eating, 39%(±3%) for emotional eating and 40%(±3%) for external eating. The previous study and current study both have adjusted for genetic influence from age, sex, age^2, age x sex, and age^2 x sex interactions. Current study reported significantly higher heritability in emotional and external eating. The reason this study resulted in higher heritability could be due to population size, first. This study included 2606 participants who have been recruited till 2011; however, the previous study did include only 2,144 recruited until 2008. Second, the previous study assumed that the distribution of eating behavior followed t-distribution to avoid inflated heritability estimates, although this study implemented multivariate normal distribution assumption. These theoretical differences could have led discrepancies in heritability.

2. Strength and weakness

There are some limitations in this study. First, range of eating behavior scores was quite narrow. Because of narrow range, there could possibly be falsely negatively detected markers. However, despite of this negative pressure, there was a significant SNP rs522723. This could mean the SNP has strong association than we expected. Second, this study has no comparable replication set. This study has been done in one ethnic group: Korean. However, to get rid of possibility to bring false positive association, we might need to scan associations in different genetic population which has different LD structure.

3. Public health implication

Previous studies let us know that there is genetic contribution on eating behavior. Therefore, it became clearer that motivation to eat is not simply controllable trait. If more genetic caused for eating behavior so as to account for more contribution, and cost of genetic test for the SNP rs522723 meets demands for preventing obesity caused by eating behavior, then we could implicate this result to indicate obesity-related problems. In assumption, a person with emotional eating behavior problem, may prevent obesity through psychological treatment, not just simple reduction of energy consumption..

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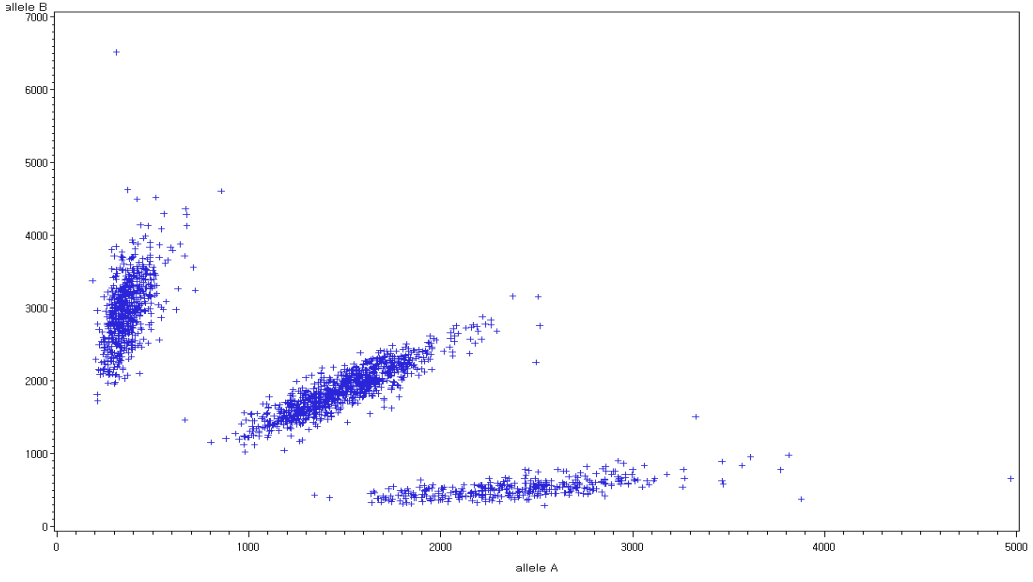
Supplement



Supplementary figure 1 scatter plots of SNP rs522723