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Two SNPs Associated with Type 2 Diabetes and Obesity at Melanocortin-4 Receptor Gene Loci Exhibited High Fst Values and Natural Selection

Issei Yoshiuchi*

Departments of Genetics, Diabetes Mellitus, and Medicine, Yoshiuchi Medical Diabetes Institute, Japan

Abstract

Background: Human adaptation to environmental changes in food supply, lifestyle, and geography may have initiated the selection of genes associated with the metabolism of glucose, lipids, carbohydrates, and energy. Obesity is significantly associated with type 2 diabetes mellitus, metabolic syndrome, hypertension, stroke, and cardiovascular diseases. The worldwide prevalence of obesity and type 2 diabetes is increasing steadily. Obesity and type 2 diabetes are highly heritable diseases that cause serious health problems. The melanocortin-4 receptor gene is one of the major obesity genes, and common genetic variations near the melanocortin-4 receptor gene were found to be associated with obesity, type 2 diabetes, and insulin resistance. We aimed to uncover evidence of selection at melanocortin-4 receptor gene loci using single-nucleotide polymorphisms (SNPs) associated with type 2 diabetes and obesity.

Methods: We analyzed melanocortin-4 receptor gene loci in HapMap populations to detect selection using a 3-step test: Wright's F-statistics (Fst) as a measure of population differentiation, the long-range haplotype (LRH) test to obtain evidence of positive selection by testing haplotypes, and integrated haplotype score (iHS).

Results: We observed one body mass index (BMI)-associated SNP (rs7227255) and one type 2 diabetes-associated SNP (rs11873305) that exhibited high Fst values, and showed high population differentiation and natural selection at the melanocortin-4 receptor gene loci.

Conclusion: This is the first report that SNPs associated with type 2 diabetes and obesity exhibited high Fst values in one-gene loci. Most of the SNPs associated with type 2 diabetes and obesity that were reported did not demonstrate high Fst values. Our findings are important for clinical medicine. We suggest that melanocortin-4 receptor agonists may be useful drugs for the treatment of obesity and type 2 diabetes, and that further studies should examine the adaptive evolution of obesity, type 2 diabetes, and insulin resistance genes.

Keywords: Natural selection ; MC4R; Obesity; Type 2 diabetes

Introduction

Obesity is one of the more significant diseases that comprise metabolic syndrome, and is a serious problem that results in morbidity and mortality worldwide. Obesity and type 2 diabetes were reported to be highly heritable diseases and genetic contribution to obesity is approximately 40–70% [1,2]. Obesity is associated with many diseases, including type 2 diabetes, hypertension, stroke, cardiovascular diseases, and several cancers. The worldwide prevalence of obesity is increasing by the day and has more than doubled since 1980. According to the 2008 World Health Organization (WHO) Report, 1.5 billion adults were overweight, and of these, over 200 million men and nearly 300 million women were obese. Furthermore, 33.8% of the population in the US in 2007-2008 was obese [3].

The past 10,000 years, also known as the Holocene epoch, have witnessed significant developments in plant and animal domestication [4]. At the end of the last Ice Age approximately 10,000 years ago, humans underwent a significant lifestyle change from being hunters and gatherers to being farmers [4]. Natural selection allows adaptations to changes in human lifestyle and geography. Throughout the past 10,000 years, human genes associated with many important diseases, including those affiliated with infection [5,6] and diabetes [7,8], may have experienced adaptive evolution. Human adaptations to environmental changes in food supply, lifestyle, and geography may have pressured the selection of genes associated with the metabolism of glucose [7,8], lipids and energy.

The melanocortin-4 receptor is associated with energy expenditure, food intake, and obesity [9], and is primarily expressed in the brain [10], but not expressed in the adrenal cortex and melanocytes [10]. Common variations near the melanocortin-4 receptor (*MC4R*) gene were found to be associated with obesity, type 2 diabetes, and insulin resistance [11-15]. Mutations of the *MC4R* gene were found to be associated with severe obesity, hyperphagia, and severe hyperinsulinemia [16].

In this study, we analyzed the *MC4R* gene loci to detect selection in HapMap populations [17] using a 3-step test that included Wright's F-statistics (Fst) as a measure of population differentiation [18,19], the long-range haplotype (LRH) test to obtain evidence of positive selection by testing haplotypes [6], and the integrated haplotype score (iHS), which is a statistic based on the differential levels of linkage disequilibrium (LD) surrounding a positively selected allele compared with the background allele [20].

***Corresponding author:** Issei Yoshiuchi, Departments of Genetics, Diabetes Mellitus, and Medicine, Yoshiuchi Medical Diabetes Institute, 2-16-41 Kamakurayama, Kamakura City, Kanagawa 248-0031, Japan, Tel: +81-467-31-8869; Fax: +81-467-31-8869; E-mail: yoshiuchi@m9.dion.ne.jp

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We observed one body mass index (BMI)-associated single-nucleotide polymorphism (SNP) (rs7227255) and one type 2 diabetes mellitus-associated SNP (rs11873305) that exhibited high Fst values and demonstrated high population differentiation and natural selection at the *MC4R* gene loci. This is the first report that these type 2 diabetes- and obesity-associated variants exhibited high Wright's F-statistics (Fst) values and showed natural selection in one-gene loci. Most of the SNPs that were associated with type 2 diabetes and obesity were reported not to demonstrate high Fst values.

Subjects and Methods

Study populations

For Wright's Fst analyses [18,19], we used genotype data from 4 human HapMap populations, including Utah residents with Northern and Western European ancestry (CEU), Yoruba in Ibadan, Nigeria (YRI), Han Chinese in Beijing (CHB), and Japanese in Tokyo (JPT), from release 28 of HapMap data [17] (Supplementary Table 1).

For the LRH test [6], we used phased haplotype data of 60 CEU, 60 YRI, and 90 East Asian (including those from the CHB and JPT groups) individuals from release 24 of HapMap data [17].

For the iHS test [20], we used 3 HapMap populations from release 21 of the HapMap dataset [17], including CEU, YRI, and East Asian individuals.

Statistical evaluation

We examined a 3-step test, Wright's Fst [18,19], the LRH test [6], and the iHS test [20] to detect selection in the *MC4R* loci using HapMap populations [17].

Wright's fixation index, Fst [18,19], a measure of population differentiation, was calculated for the 4 HapMap populations with Arlequin integrated software (version 3.1; http://cmpg.unibe.ch/software/arlequin3/) [21]. We tested four BMI-associated SNPs (rs6567160, rs571312, rs17782313, and rs7227255 [11,13,14]), one type 2 diabetes, waist circumference, and insulin resistance-associated SNP (rs12970134 [12]), and one type 2 diabetes-associated SNP (rs11873305 [15]), from *MC4R* loci (Supplementary Table 1). In previous studies that utilized Fst analysis, the global Fst for the 95th percentile of over 2,911,292 markers was 0.365 [22] in 4 HapMap populations, including that of CEU, YRI, CHB, and JPT populations [17], and the average Fst value was ~0.15 [23-26]. We compared the 6 Fst values with the 95th percentile of the Fst distribution (>0.365) [22].

The LRH test [6], which was performed to obtain evidence of positive selection by testing haplotypes, was performed with SWEEP (version 1.1: http://www.broadinstitute.org/mpg/sweep/index.html) [6]. Using the LRH test, specifically, the relative extended haplotype homozygosity (REHH) method [6], we scanned not only the 6 associated SNP regions but also the *MC4R* loci (Chr. 18, locations 55,980,000–56,500,000), and analyzed these loci with the complete chromosome 18 in each population.

The iHS, a statistic based on the differential levels of LD surrounding a positively selected allele as compared to the background allele, was calculated using the Haplotter web tool (http://haplotter.uchicago.edu/), which was accessed in January 2010 [20]. In the iHS test, an extreme positive score (iHS > 2.5) and an extreme negative score (iHS < -2.5) represent the highest 1% of the distribution of scores for all SNPs [20]. Furthermore, extreme positive scores ([iHS] 2.5) indicate recent positive selection at a locus [20]. The iHS has moderate power to detect a selection at intermediate frequency (50–80%), but low power to detect a selection at high frequency (>80%) or low frequency (<30%) [27]. we tested the SNPs of the *MC4R* loci (Chr. 18, locus 55,980,000-56,500,000), and all iHS values were standardized [20].

Results

Fst analysis

We tested four BMI-associated SNPs [11,13,14], one type 2 diabetes, waist circumference, and insulin resistance-associated SNP [12], and one type 2 diabetes-associated SNP [15], from the *MC4R* loci with Fst analysis (Table 1, Supplementary Table 1). We compared the 6 Fst values with the upper 95% quantile of the distribution (>0.365) [22] and found the highest Fst values in one BMI-associated SNP (rs7227255:0.42) and one type 2 diabetes-associated SNP (rs11873305:0.41) (Table 1). The two SNPs (rs7227255 and rs11873305) with the highest Fst values were located in a region approximately 9-16 kb upstream of *MC4R*. The other four SNPs with low Fst values were located in a region approximately 150-210 kb downstream of *MC4R*. In particular, the rs7227255 C risk allele of the MC*4R* loci is reportedly associated with BMI. Accordingly, the rs7227255 C risk allele may be thrifty genotype [28]. These findings are very interesting and the first report that both variants associated with type 2 diabetes and obesity exhibited high Fst values and showed natural selection in one-gene loci. Most of the associated SNPs were reported not to demonstrate high Fst values [22,27], but these results specifically presented associated SNPs themselves showed high Fst values.

LRH test

We found selection in the regions approximately 192-196 kb upstream, approximately 29-80 kb downstream, and approximately 134-145 kb downstream of *MC4R* in the CEU population (REHH percentile: 99.9) (Table 2). The gene nearest to these regions is *MC4R*, and these regions may be regulatory regions of *MC4R*. It has been reported that regions that are several hundred kilobases away from a gene could be regulatory regions [29]. These regions could be the actual target of selection in *MC4R* loci.

Gene	Associated SNP	Chr	Position	Risk/non risk	Associated phenotypes	Fst*
MC4R	rs6567160	18	55,980,105	G/A	BMI	0.02
	rs571312		55,990,739	A/C	BMI	0.05
	rs17782313		56,002,067	C/T	BMI	0.01
	rs12970134		56,035,730	A/G	Type 2 diabetes/ Insulin resistance/ Waist Circumference	0.03
	rs11873305		56,200,172	A/C	Type 2 diabetes	0.41
	rs7227255		56,206,701	C/T	BMI	0.42

*The reported 95% quantile of over 2,911,292 markers is 0.365 [22].

Table 1: Fst values of 6 associated SNPs at MC4R loci.

Population	Associated SNP	Start Base	Length	Haplotype frequency	REHH	REHH percentile	REHH P-value
CEU		56045103	9888	0.308	8	99.9th	0.012
		56109995	50411	0.308	9	99.9th	0.010
		56383434	3202	0.167	31	99.9th	0.007
YRI	rs65670 G risk and rs571312 C non-risk alleles	55964628	26121	0.125	56	99.9th	0.013

ASN: East Asian including CHB and JPT

Table 2: The LRH test at the MC4R loci (REHH percentile ≥ 99.9th).

Population	Number of SNPs with [iHS] > 2.5 (Total number of SNPs) Max [iHS] (rs number: Position)
CEU	0 (514) 2.19 (rs11660003:56374946)
YR	5 (625) 2.89 (rs12326252:56483425)
ASN	6 (470) 3.01 (rs2226: 56435992)

Table 3: Standardized [iHS] scores at the MC4R loci.

In the YRI population, we found that the core haplotype with a BMI risk allele (rs65670 G) and a BMI non-risk allele (rs571312 C) exhibited selection (REHH percentile: 99.9) (Table 2).

iHS test

Extreme positive scores ([iHS] > 2.5) indicate recent positive selection at a locus [20]. We observed that the highest [iHS] score of the SNP rs12326252 was 2.89 in the YRI population (Table 3), and the highest [iHS] score of the SNP rs2226 was 3.01 in East Asian populations (Table 3). These SNPs (rs12326252 and rs2226) were located at a region approximately a few hundred kilobases upstream of *MC4R* (Table 3). These SNPs were not linked with obesity, type 2 diabetes, or insulin resistance but may be linked with unknown phenotypes associated with *MC4R*.

Discussion

Charles Darwin originally defined natural selection in *On the Origin of Species*, published in 1859 [30]. Various adaptations to changes in food supply, lifestyle, and geography may have initiated the selection of genes associated with metabolism of glucose [7,8], lipids, carbohydrates, and energy. During the past 10,000 years of human history, some important genes may have been the target of selection, becoming more differentiated to allow human populations to adapt and survive in different environments with different geography and different food supply [8]. The selected genes, therefore, could be historical footprints to our modern genome.

Evaluating gene selection can be both complex and difficult, but efforts to discover selection in genes associated with obesity, type 2 diabetes, and insulin resistance could be extremely useful in clinical medicine for the treatment of obesity and diabetes based on the genotypes of selected obesity and diabetes genes. The prevalence of obesity and diabetes is increasing steadily, and both conditions can lead to the development of serious complications. However, differences in the prevalence of obesity among ethnic groups may be not only because of genetic factors, but also because of environmental factors.

According to the 2008 WHO Report, worldwide prevalence of obesity has increased greatly in the past 3 decades and has more than doubled since 1980. Furthermore, obesity is reported to be a highly heritable disease [1,2]. According to the 2007-2008 US National Health and Nutrition Examination Survey (NHANES), the prevalence of obesity among US adults showed that 32.8% of non-Hispanic whites, 44.1% of non-Hispanic blacks, and 39.3% of Mexican Americans were obese [3]. These findings demonstrated that there was a difference in the prevalence of obesity between racial and ethnic groups.

In particular, *MC4R* is one of the major obesity genes [11-14,16,31,32], and is associated with energy expenditure, food intake, and BMI [9]. *MC4R* is a seven transmembrane G-protein coupled receptor and is highly expressed in the hypothalamus [9,10]. *MC4R*-deficient mice showed a maturity onset obesity syndrome associated with hyperphagia, hyperinsulinemia, and hyperglycemia [32]. The melanocortin signaling plays important roles in the control of appetite and weight. Frameshift mutations in the *MC4R* gene were found to be associated with a dominant form of obesity [16,31]. Common variations near *MC4R* were found to be associated with obesity, type 2 diabetes, and insulin resistance [11-15].

In this study, we detected the highest Fst values in one BMI-associated SNP (rs7227255; Fst=0.42) and one type 2 diabetes-associated SNP (rs11873305; Fst=0.41) (Table 1), and demonstrated high population differentiation and natural selection at the *MC4R* gene loci in HapMap populations. The two SNPs (rs7227255 and rs11873305) were located in a region approximately 9-16 kb upstream of *MC4R*. We suggest that the rs7227255 C risk allele may be a thrifty genotype [28]. In a phylogenetic analysis, *MC4R* was reported to be subject to strong purifying selection [33].

In conclusion, these findings are very meaningful for clinical medicine and the first report that two SNPs associated with type 2 diabetes and obesity exhibited high Fst values and natural selection at the *MC4R* one-gene loci. We propose that the adaptive evolution of the genes associated with obesity, type 2 diabetes, and insulin resistance, especially genes involved in the *MC4R* pathway, such as leptin, leptin receptor, agouti-related protein (AGRP), and pro-opiomelanocortin (POMC), needs to be investigated further. Furthermore, we suggest that multiple genetic polymorphisms at the *MC4R* gene loci may lead to differences in resistance to obesity among ethnic groups, based on their current food supply and lifestyles.

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The protocol for this research project has been approved by an Ethics Committee of Yoshiuchi Medical Diabetes Institute within which the work was undertaken and that it conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000).

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