RESEARCH NOTE

Polymorphisms in *FTO*, *TMEM18* and *PCSK1* are associated with BMI in southern Chinese population

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Introduction

Obesity is a major health problem in the world, and is defined as excessive fat accumulation and quantified by body mass index (BMI). Genetic factors play a key role in obesity. In the last few years, genomewide association studies (GWAS) have produced a number of novel genes and polygenic variants associated putatively with obesity. Common variants at *FTO*, *INSIG2* and *MC4R* loci were previously reported to be associated with fat mass, weight and obesity in several populations (Sandholt *et al.* 2010). Yanagiya *et al.* (2007) revealed that genetic variations in *MTMR9* may confer a predisposition towards obesity. *PCSK1* was also firmly placed on the short list of genes reproducibly associated with common obesity (Benzinou *et al.* 2008). A meta-analysis of 15 GWAS performed by the GIANT consortium on total 32,387 individuals of European ancestry discovered that six previously unreported loci in or near *KCTD15*, *SH2B1*, *GNPDA2*, *TMEM18*, *NEGR1* and *MTCH2* are associated with BMI (Willer *et al.* 2009).

In this study, we attempt to examine the association between BMI and the genes mentioned here by genotyping the previously reported 11 single-nucleotide polymorphism (SNP) in southern Chinese.

Materials and methods

Subjects

This study included 1560 southern Chinese individuals (288 men and 1272 women) with mean age of 49.8 ± 15.9 years (women, 49.5 ± 15.9, men, 50.9 ± 15.9), mean height of 1.57 ± 0.08 m (women, 1.55 ± 0.07, men, 1.67 ± 0.07), and mean weight of 55.7 ± 10.8 kg (women, 53.4 ± 9.3, men, 53.4 ± 9.3). A detailed description of subject ascertainment, inclusion and exclusion criteria has been described previously (Ng *et al.* 2006). BMI was calculated as weight (kg)/height^2 (m^2).

SNP selection and genotyping

Eleven SNPs from 10 candidate genes (*FTO*, *KCTD15*, *INISG2*, *SH2B1*, *PCSK1*, *MTMR9*, *GNPDA2*, *TMEM18*, *MC4R* and *NEGR1*), which have been reported to be associated with BMI, were selected for genotyping in 1560 subjects using the high-throughput mass array technology. Five percent of samples were duplicated for quality check in the genotyping process and the reproducibility rate exceeded 99.8%.

Statistical analysis

Since the raw data of BMI deviated from normal distribution, a square root transformation was conducted. Genotype–BMI association analyses were performed by linear regression, SNP being coded as 0, 1 and 2 according to genotypes. Analyses included linear regression of single SNP (adjusted with age) with BMI and multiple stepwise regressions of 11 SNPs, and age with BMI. Statistical analyses were performed using SPSS 11.5 for Windows software. A P value less than 0.05 was considered to be statistically significant.

Results and discussion

The BMI in our samples ranged from 14.97 to 37.95 kg/m^2. Kolmogorov–Smirnov test suggested that the distribution of BMI deviated from normal distribution ($P = 0.001$), but square root of BMI (sqrt BMI) followed normal distribution ($P = 0.056$) (figure 1).

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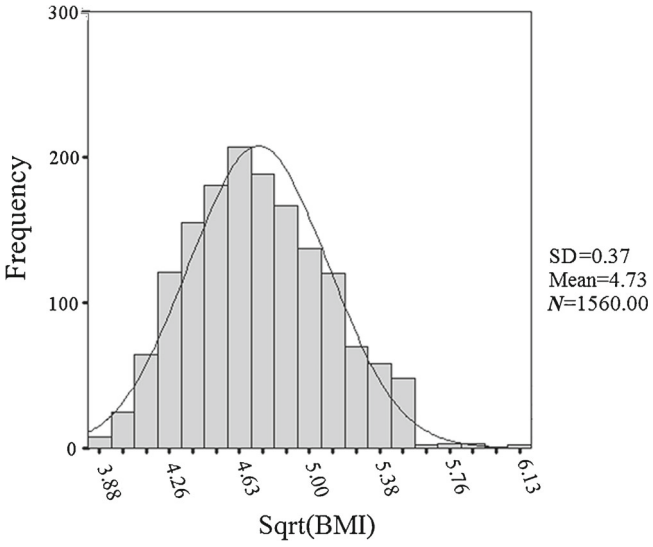

Figure 1. Histogram graph with normal curve of sqrt (BMI).

Linear regression between single SNP and age, and square root of BMI was performed (table 1). Significant partial regression coefficient were found for *FTO* rs9939609 ($P = 0.004$) and *TMEM18* rs6548238 ($P = 0.003$). *KCTD15* (rs11084753), *PCSK1* (rs6234, rs6235), *MTMR9* (rs2293855) and *NEGR1* (rs2815752) showed a weak correlation with BMI. For *FTO* rs9939609, BMI of AA (23.25 kg/m^2) and AT (22.87 kg/m^2) genotypes were significantly higher than those of TT (22.35 kg/m^2) genotype ($P = 0.010$). It seems that the A allele increases BMI in dominant model. For rs6548238 (*TMEM18*), there is significant difference in BMI between CC (22.60 kg/m^2) and TC (21.98 kg/m^2) genotype ($P = 0.015$). TT genotype had the lowest BMI (20.84 kg/m^2). Multiple stepwise regression equation of BMI on 11 SNPs and age included *FTO*, *TMEM18* and *PCSK1* genes: sqrt BMI $= 4.216 + 0.065x_1$ (rs6548238, $P = 0.007$) $+0.058x_2$ (rs9939609, $P = 0.002$) $+0.030x_3$ (rs6234, $P = 0.031$) $+0.007x_4$ (age), $R^2 = 10.4\%$.

Subgroup analyses were also performed to examine gender difference of obesity genetics. For women, apart from FTO rs9939609 ($P = 0.006$) and TMEM18 rs6548238 ($P = 0.007$), an association was found between GNPDA2 rs10938397 ($P = 0.014$) and BMI. For men, only MC4R rs17782313 was associated with BMI ($P = 0.034$). Causes for gender difference included sex-specific genetic control and small sample size of men's subgroup.

FTO, located on chromosome 16q12.2, was earliest identified as a susceptible locus for obesity. rs9939609 in *FTO* was identified to be predisposed to diabetes through an effect on BMI in a genomewide search for type 2 diabetes, and then first reported as obesity susceptibility gene (Sandholt *et al.* 2012). In two ongoing longitudinal studies, Liu *et al.* (2011) assessed the influence of rs9939609 on development of adiposity in European-American and African-American youth, and found that rs9939609 was associated with logBMI. So far, six studies have been published on rs9939609 locus of *FTO* in Chinese populations. Although two studies found no association of rs9939609 variant with obesity (Li *et al.* 2008; Yan *et al.* 2009), rs9939609 is associated with BMI in ethnic Chinese in Singapore (Tan *et al.* 2008), Chinese children (Fang *et al.* 2010), Taiwan Chinese Han populations (Chang *et al.* 2008) and Hubei Han Chinese aged from 50–70 years (Huang *et al.* 2011). Our current study successfully replicated association between *FTO* rs9939609 and BMI in southern Chinese from Hong Kong. Four meta-analyses of associations between rs9939609 and obesity / BMI have been published in recent years and *FTO* rs9939609 was significantly associated with BMI (Xi and Mi 2009; Liu *et al.* 2010; Chauhan *et al.* 2011; Li *et al.* 2012).

TMEM18-associated SNPs were first found to be associated with obesity by two GWAS (Willer *et al.* 2009; Thorleifsson *et al.* 2009). From then on, the association with obesity was confirmed on Swedish children (Almén *et al.* 2010), Greek (Rouskas *et al.* 2012) and Japanese populations (Takeuchi *et al.* 2011) and children and adolescents from Denmark and Estonia (Den Hoed *et al.* 2010). Although two previous studies found no association of *TMEM18* polymorphisms with BMI in Chinese populations (Ng *et al.* 2010; Huang *et al.* 2012), our study demonstrated that rs6548238 was associated with BMI in southern Chinese aged 18–85

Table 1. Association of 11 loci with BMI in southern Chinese population.

Gene	SNP (normal allele / risk allele)	Regression equation	R^2	P
GNPDA2	rs10938397(A/G)	$y = 4.367 + 0.023x_1 + 0.007x_2$	8.9%	0.107
FTO	rs9939609(T/A)	$y = 4.367 + 0.054x_1 + 0.007x_2$	9.2%	0.004*
INSIG2	rs7566605(G/C)	$y = 4.374 + 0.009x_1 + 0.007x_2$	8.8%	0.505
KCTD15	rs11084753 (A/G)	$y = 4.362 + 0.028x_1 + 0.007x_2$	9.0%	0.053
MC4R	rs17782313(T/C)	$y = 4.371 + 0.027x_1 + 0.007x_2$	9.0%	0.126
MTMR9	rs2293855(A/G)	$y = 4.367 + 0.025x_1 + 0.007x_2$	9.0%	0.078
NEGR1	rs2815752(C/T)	$y = 4.315 + 0.037x_1 + 0.007x_2$	9.0%	0.088
PCSK1	rs6234(C/G)	$y = 4.362 + 0.024x_1 + 0.007x_2$	9.1%	0.080
PCSK1	rs6235(G/C)	$y = 4.361 + 0.026x_1 + 0.007x_2$	9.1%	0.054
SH2B1	rs7498665(G/A)	$y = 4.356 + 0.014x_1 + 0.007x_2$	8.8%	0.544
TMEM18	rs6548238(T/C)	$y = 4,248 + 0.071x_1 + 0.007x_2$	9.4%	0.003*

x_1(SNP), x_2(age), * $P < 0.05$.

years. In addition, a study on Chinese children (10–20 years aged boys) from Shanghai also validated that rs6548238 in *TMEM18* has an association with obesity-related indices during puberty (Wang *et al.* 2012). A recent research has shown that *TMEM18* plays a regulatory role in human adipocyte differentiation (Bernhard *et al.* 2013).

PCSK1 encodes prohormone convertase 1/3 which can convert inactive prohormones into biologically active peptide hormones. Loss-of-function mutations in *PCSK1* give rise to monogenic obesity (Jackson *et al.* 1997). Benzinou *et al*. (2008) initially uncovered rs6232 and rs6234–rs6235 in *PCSK1* strongly associated with obesity risk among adults and children in European populations. Association of rs6232 and rs6235 with obesity, and BMI were also replicated in European (Kilpeläinen *et al.* 2009; Sandholt *et al.* 2010; Rouskas *et al.* 2012) and Mexican populations (Villalobos *et al.* 2012). Here, our study demonstrated that rs6234 was associated with BMI in Chinese.

Our present study demonstrated that *FTO*-rs9939609, *TMEM18*-rs6548238 and *PCSK1*-rs6234 polymorphisms are significantly associated with BMI in southern Chinese population. However, we are unable to replicate the associations for other loci; due to small sample size and limited statistical power.

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