

## Original Article



# Genome Wide Association Study: Searching for Genes Underlying Body Mass Index in the Chinese\*

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## Abstract

**Objective** Obesity is becoming a worldwide health problem. The genome wide association (GWA) study particularly for body mass index (BMI) has not been successfully conducted in the Chinese. In order to identify novel genes for BMI variation in the Chinese, an initial GWA study and a follow up replication study were performed.

**Methods** Affymetrix 500K SNPs were genotyped for initial GWA of 597 Northern Chinese. After quality control, 281 533 SNPs were included in the association analysis. Three SNPs were genotyped in a Southern Chinese replication sample containing 2 955 Chinese Han subjects. Association analyses were performed by Plink software.

**Results** Eight SNPs were significantly associated with BMI variation after false discovery rate (FDR) correction ( $P=5.45 \times 10^{-7}$ - $7.26 \times 10^{-6}$ , FDR  $q=0.033$ - $0.048$ ). Two adjacent SNPs (rs4432245 & rs711906) in the eukaryotic translation initiation factor 2 alpha kinase 4 (EIF2AK4) gene were significantly associated with BMI ( $P=6.38 \times 10^{-6}$  &  $4.39 \times 10^{-6}$ , FDR  $q=0.048$ ). In the follow-up replication study, we confirmed the associations between BMI and rs4432245, rs711906 in the EIF2AKE gene ( $P=0.03$  &  $0.01$ , respectively).

**Conclusion** Our study suggests novel mechanisms for BMI, where EIF2AK4 has exerted a profound effect on the synthesis and storage of triglycerides and may impact on overall energy homeostasis associated with obesity. The minor allele frequencies for the two SNPs in the EIF2AK4 gene have marked ethnic differences between Caucasians and the Chinese. The association of the EIF2AK4 gene with BMI is suggested to be 'ethnic specific' in the Chinese.

**Key words:** Body mass index; Genome wide association; EIF2AK4; Replication

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## INTRODUCTION

Obesity is becoming a global health problem, affecting people in both developing and developed countries<sup>[1-2]</sup>. Approximately 250 million adults, nearly 7% of the world adult population, are estimated to suffer from obesity<sup>[3-4]</sup>. According to statistical data (2004) from the Ministry of Health of China, approximately 7.1% of the total 1.3 billion population are affected with obesity, and the prevalence of obesity in adults in large cities account for 12.3%. Body mass index (BMI), a key index of body composition, is widely used for defining morbid obesity and assessing risks to cardiovascular disease and type 2 diabetes<sup>[5-6]</sup>. BMI is a complex quantitative trait, determined by multiple genetic and/or environmental factors<sup>[7]</sup>. The estimated heritability of BMI ranges from 0.50 to 0.90<sup>[8-9]</sup>.

Many quantitative trait loci or candidate genes underlying BMI variation have been identified using genome wide linkage (GWL) scans or candidate gene approaches<sup>[10-29]</sup>, though both of these methods have their own limitations. GWL studies have identified many genomic regions associated with BMI<sup>[10,12-18,20-22,29]</sup>, however, few of these regions have been replicated in other populations. Genomic regions identified in GWL scans are typically fairly large and few follow-up fine mapping studies have been successfully pursued. Candidate gene association mapping approaches are considered more powerful than GWL studies, nevertheless, the selection of these genes is based on prior knowledge of gene function so these approaches are not designed to identify novel genes influencing BMI, and the number of genes successfully tested is limited.

Recent technological advances in single-nucleotide polymorphism (SNP) genotyping in conjunction with increased knowledge of linkage disequilibrium (LD) patterns in major human populations, as revealed by the HapMap project, have enhanced the practicality of assessing the entire human genome by assaying hundreds of thousands of SNPs simultaneously. Consequently, genome wide association (GWA) study is becoming an important approach toward identifying common variants associated with complex diseases or quantitative traits. Recent GWA studies have identified several significant genes associated with obesity-related phenotypes, primarily on BMI in Caucasians<sup>[30-35]</sup>. For example, our group conducted a

GWA study for BMI and fat mass in Caucasians and identified a novel gene, *CTNBL1*, which may play an important role in the development of obesity<sup>[30]</sup>.

Ethnic disparity in the genetic background has been proposed as an important factor contributing to the variation of obesity-related traits across different populations, thereby justifying genetic studies in distinct ethnic populations (details in Discussion). To the best of our knowledge, except for one GWA study for BMI conducted in Koreans<sup>[36]</sup> and the other conducted in the Japanese<sup>[37]</sup>, no more GWA studies particularly for BMI on population based samples have been previously performed in the Chinese, the largest population in the world. In order to identify novel genes and search for potential ethnic-specific genes for BMI in the Chinese, we performed a modest GWA study in 597 unrelated Northern Chinese adults using a highly dense Affymetrix 500K SNP array examining about 500 000 SNPs and a follow-up replication study in an independent sample of 2 955 unrelated Southern Chinese.

## MATERIALS AND METHODS

### Subjects

The study was approved by the Institutional Review Board or Research Administration of the involved institutions. Signed informed-consent documents were obtained from all study participants before they were enrolled in the study.

**The Northern Chinese Sample for GWA** The study was initially performed with a GWAS discovery stage for SNPs of potential significance for bone mineral density in a Chinese Han sample in Xi'an City and the surrounding areas of Northern China. Subjects with serious metabolic diseases (diabetes, hypo-and hyper-parathyroidism, hyperthyroidism, etc.) or chronic use of drugs affecting metabolism were excluded. The detailed procedure of exclusion was presented by a previous study<sup>[38]</sup>. The sample included 258 men and 339 women. Weight was measured on electronic scales to the nearest 0.1 kg and height was measured to the nearest 0.1 cm with a wall-mounted stadiometer with subjects wearing light clothing and no shoes. BMI was calculated as body weight (in kilograms) divided by the square of height (in meters).

**The Southern Chinese Sample for Replication** These unrelated subjects were randomly selected from the extended database of an unrelated sample

in the Changsha City and the surrounding area located in Southern China. The detailed sampling method can be found by a previous study of our group<sup>[6]</sup>. A total of 2 955 Chinese Han subjects (1 518 women and 1 437 men) aged 19-88 years were included in the final replication study. The basic characteristics of all the studied subjects are presented in Table 1.

### Genotyping

#### **Affymetrix 500K SNPs in 597 Chinese in Northern China**

Genomic DNA was extracted from whole human blood using a commercial isolation kit (Gentra systems, Minneapolis, MN, USA) according to the protocols of the kit. For each sample, genotyping with GeneChip® Human Mapping 500K set containing 250K Nsp array and 250K Sty array (Affymetrix, Santa Clara, CA, USA) was performed using the standard protocols recommended by the manufacturer. Briefly, for each array, 250 ng of DNA was digested with restriction enzyme (NspI or StyI) and ligated to adapters. A single PCR primer that recognizes the adapter sequence was used to amplify the ligated product. The amplified DNA (200-1100 bp) was fragmented into approximately 50 bp size, then labeled with biotin and hybridized to the arrays. After 16-18 h of hybridization, the arrays were washed with Wash Buffer A (6×SSPE, 0.1% Tween20) and Wash Buffer B (0.6×SSPE, 0.1% Tween20), in turn, on an Affymetrix Fluidics Station FS450. Then they were stained with the Streptavidin Phycoerythrin (SAPE, 10 µg/mL) and the signals were amplified with Anti-streptavidin antibody. The stained arrays were scanned with an Affymetrix GeneChip® 3000 7G scanner at 0.7 µm solution and

generated relevant signal images. SNPs genotypes from the scanned images were extracted using GCOS and GTYPE software (Affymetrix).

Quality control procedures were as follows. First, only samples with a minimum of 95% call rate were included. The final mean BRLMM call rate of the entire sample reached a high level of 99.02%. Second, out of the initial full-set of 500 568 SNPs, we discarded: 1) SNPs with a call rate <90% in the total sample ( $n=54\ 845$ ); 2) those deviating from Hardy-Weinberg equilibrium (HWE) ( $P<0.001$ ,  $n=22\ 002$ ); and; 3) those having a minor allele frequency (MAF) <0.05 in the total sample ( $n=142\ 188$ ). Therefore, 281 533 SNPs were available for the subsequent analyses.

#### **Replication Genotyping in 2 955 Subjects in Southern China**

Three selected SNPs were genotyped for replication study in the unrelated Southern Chinese sample. Genotyping of subjects was performed using a primer extension method with MALDI-TOF mass spectrometry for multiplexed genotyping of SNPs on a MassARRAY system as suggested by the manufacturer (Sequenom, Inc., San Diego, CA). The method was described by Braun et al.<sup>[39]</sup>. The SNP genotyping success rate was 97% and the duplicate concordance rate was 99%. The three SNPs were all in HWE ( $P>0.10$ ), and the MAFs of the SNPs were consistent with the MAFs in initial GWA (Table 3).

### Statistical Analyses

#### **Initial GWA**

The two significant covariates, gender and age, were used to adjust raw BMI values for subsequent analyses. Then HelixTree 5.3.1 (Golden Helix, Bozeman, MT) was used to perform genotypic association analyses and haplotype association analyses. The linkage disequilibrium (LD) [standardized  $D'$  ( $D/D_{max}$ )] patterns for genes of interest were analyzed and plotted using the Haploview program<sup>[40]</sup>. Genotypic association analyses were used to compare the difference of mean BMI values among three genotypic groups for each SNP. Haplotype association or block association detected the different mean BMI values among the haplotype groups within haplotype blocks. We also performed marker-marker interaction analyses for those significant markers identified in single SNP analyses using the two-loci genetic association analysis implemented in HelixTree. Marker-marker interaction was used to compare difference of mean BMI values among the new categorical variable formed by the combination of the two markers.

**Table 1.** Basic Characteristics of the Study Subjects

Study	Trait	Total	Female	Male
GWA Study	Sample size	597	339	258
	Age (years)	70.4±7.4	69.7±7.8	71.1±6.8
	Height (cm)	160.8±8.9	155.5±6.3	167.8±6.9
	Weight (kg)	59.4±11.4	56.3±10.7	63.6±11.1
	BMI (kg/m <sup>2</sup> )	22.9±3.8	23.2±4.0	22.5±3.4
Replication Study	Sample size	2955	1518	1437
	Age (years)	33.1±14.5	35.4±15.7	30.5±12.5
	Height (cm)	163.7±7.9	158.1±5.3	169.6±5.6
	Weight (kg)	58.6±10.2	53.4±8.1	64.0±9.3
	BMI (kg/m <sup>2</sup> )	21.8±3.1	21.4±3.2	22.2±2.9

Multiple testing is a perplexing issue in GWA studies. As Bonferroni adjustment for multiple-testing in a GWA study is usually considered to be extremely conservative, we used QVALUE software developed by Storey and Tibshirani<sup>[41]</sup> to calculate a FDR (false discovery rate) based  $q$  value to measure the statistical significance at the genome wide level for the association results. The cutoff for significant association at the genome wide level was set at FDR  $q$  value  $\leq 0.05$ .

To detect spurious association results that may be brought by potential population stratification, we used STRUCTURE 2.2 software to investigate the potential substructure of our sample. The program uses a Markov chain Monte Carlo (MCMC) algorithm to cluster individuals into different cryptic sub-populations on the basis of multi-locus genotype data<sup>[42]</sup>. We performed an independent analysis under the assumed number of population strata  $k=2$  and a set of 1 000 un-linked markers randomly selected from about 280 000 eligible SNPs of the whole genome. We also used the method of genome control to detect potential population stratification of this sample. It can estimated the inflation factor ( $\lambda$ ) based on the genome-wide SNP information. We further performed the principal component analysis (PCA) implemented in EIGENSTRAT to guard against possible population stratification.

In order to analyze and predict the function of the significant SNPs identified, and other interesting SNPs, we utilized the FASTSNP program to provide up-to-date information about known and potential functional effects of SNPs<sup>[43]</sup>.

**Replication Analyses** First, we adjusted age and sex for the raw BMI values, then transformed them into normal distribution with box-cox transformation. Plink software<sup>[44]</sup> was used to perform the general genotype-based association test.

Finally, the  $P$  values for significant SNPs from initial GWA study and the follow-up replication study were combined using Fisher's method<sup>[45]</sup> to quantify the overall evidence for association with BMI variation. It is a 'meta-analysis' technique for combining the results from a variety of independent tests bearing upon the same overall hypothesis as if in a single test. Fisher's method combines extreme value probabilities from each test, called ' $P$ -values', into one test statistic ( $\chi^2$ ) having a chi-square distribution using the formula  $\chi^2_{2k} = -2 \sum_{i=1}^k \log_e(p_i)$ , whereas  $k$  is the degrees of freedom of the  $\chi^2$  statistic combining  $p_i$  from the study<sup>[45]</sup>.

## RESULTS

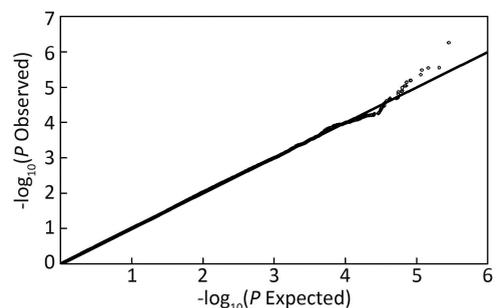
### Initial GWAS Study

Using about 280 000 eligible SNPs, we examined the quantile-quantile (Q-Q) plot for the distribution of  $P$  values involving all SNPs tested in our sample (Figure 1). We observed a fraction of SNPs associated with BMI compared to that expected  $P$  values based on chance alone. These results indicate that the most strongly associated SNPs are likely to have true associations with BMI.

The SNPs with significant association signals in our initial association analyses, after FDR correction ( $q$  value  $\leq 0.05$ ), are summarized in Table 2. Eight SNPs in seven genes showed significant association signals with BMI ( $P=5.45 \times 10^{-7}$ - $7.26 \times 10^{-6}$ , FDR  $q=0.033$ - $0.048$ ). The most significant SNP is rs4633, located in the exon of the catechol-O-methyltransferase (COMT) gene ( $P=5.45 \times 10^{-7}$ , FDR  $q=0.033$ ). Two adjacent SNPs (rs4432245 & rs711906) in the eukaryotic translation initiation factor 2 alpha kinase 4 (EIF2AK4) gene were significantly associated with BMI ( $P=4.38 \times 10^{-6}$  and  $6.39 \times 10^{-6}$ , respectively, FDR  $q=0.048$ ).

### Replication Study

We further genotyped SNPs rs4432245, rs711906, and rs4633 in an independent Southern Chinese sample. We selected these three SNPs because: 1) of the 8 SNPs which had significant  $q$ -value, the three SNPs in these two genes might have some known direct or indirect function in the pathogenesis of obesity; 2) genotyping budget for replication was limited. Two SNPs, rs4432245 and rs711906 in EIF2AK4 gene, showed significant association with BMI ( $P=0.03$  &  $0.01$ , respectively) under



**Figure 1.** Quantile-quantile (Q-Q) plots for BMI association. The Y-axis is the-LOG<sub>10</sub>(p) values for the GWA study SNPs and the X-axis is the-LOG<sub>10</sub>(p) values expected under the null distribution for the GWA study SNPs.

genotypic test model. However, no significant association ( $P=0.52$ ) between rs4633 and BMI was detected.

Combined  $P$  values from the initial GWA study and the replication study for rs4432245 and rs711906 in EIF2AK4 gene ( $P=1.12\times 10^{-6}$  and  $2.21\times 10^{-6}$ , respectively) supported the above significant associations between these SNPs and BMI.

### Analyses within EIF2AK4 Gene

Table 3 presents information for all analyzed SNPs in the EIF2AK4 gene, and their single-SNP association signals with BMI. Among the 14 SNPs analyzed in the EIF2AK4 gene only rs4432245 and

rs711906, located at introns 36 and 37 respectively, were significantly associated with BMI at the genome-wide level; moreover they were in strong LD. rs711906, with a polymorphic G→A nucleotide change, has a MAF of 47.0% according to our own data and 48.9% according to the HapMap CHB database (Table 3). Individuals having one or two copies of the 'G' allele of rs711906 had a BMI that was, on average, 0.9 kg/m<sup>2</sup> lower than that of non-carriers of G (Figure 2A). But in replication studies, individual having one or two copies of the 'G' allele of rs711906 had a BMI that was, on average, 0.4 kg/m<sup>2</sup> higher than that of non-carriers of G (Figure 2B).

**Table 2.** Top Eight SNPs Showing Significant Evidence for Associations with BMI (FDR  $q$  values $\leq 0.05$ ) in GWA Analyses

dbSNP ID	Chromosome Position	Physical Position	Associated Gene <sup>A</sup>	Role	Allele	MAF <sup>B</sup>	MAF <sup>C</sup>	$P$ Value	$q$ Value
rs4633	22q11.21	18330235	COMT	Exon3	T/C	0.254	0.233	$5.45\times 10^{-7}$	0.033
rs3213523	22q13.32	48131411	FLJ44385	Upstream	C/T	0.352	0.422	$2.75\times 10^{-6}$	0.048
rs1438168	3p25.3	10412280	ATP2B2	Intron4	G/A	0.409	0.389	$2.82\times 10^{-6}$	0.048
rs10904363	10p15.1	4910413	AKR1CL2	Intron5	C/G	0.417	0.356	$3.24\times 10^{-6}$	0.048
rs711906	15q15.1	38112983	EIF2AK4	Intron37	A/G	0.470	0.489	$4.38\times 10^{-6}$	0.048
rs4432245	15q15.1	38111773	EIF2AK4	Intron36	C/T	0.481	0.489	$6.39\times 10^{-6}$	0.048
rs7623901	3q13.33	123011701	IQCB1	Intron5	T/C	0.053	0.056	$6.41\times 10^{-6}$	0.048
rs12496318	3q13.33	123044190	EAF2	Intron1	T/G	0.054	0.056	$7.26\times 10^{-6}$	0.048

**Note.** A. Abbreviations: COMT (catechol-O-methyltransferase), EIF2AK4 (eukaryotic translation initiation factor 2 alpha kinase 4), FLJ44385 (uncharacterized), ATP2B2 (plasma membrane Ca(2+)-ATPase), AKR1CL2 (aldo-keto reductase family 1, member C-like 2), IQCB1 (IQ motif containing B1), EAF2 (ELL associated factor 2); B. Minor allele frequency calculated in initial GWA sample; C. Minor allele frequency reported for the Chinese or Asians in the public database such as HapMap or dbSNP.

**Table 3.** Analyzed SNPs in EIF2AK4 Gene for the GWA Sample

Associated Gene	dbSNP	Physical Position	Role	Allele <sup>A</sup>	MAF <sup>B</sup>	MAF <sup>C</sup>	$P$ Value <sup>D</sup>	FDR $q$ Value <sup>E</sup>
EIF2AK4 (15q15.1)	rs534757	38014169	Intron 1	T/C	0.167	0.144	0.624 814	NS
	rs518770	38017564	Intron 1	T/C	0.166	0.144	0.677 616	NS
	rs489508	38033071	Intron 4	T/C	0.016	0.022	0.484 009	NS
	rs16970033	38033092	Intron 4	A/G	0.195	0.211	0.377 959	NS
	rs16970035	38033128	Intron 4	C/A	0.284	0.211	0.432 093	NS
	rs8041785	38039033	Intron 6	G/A	0.46	0.456	0.469 773	NS
	rs7174767	38057092	Intron 12	T/C	0.232	0.267	0.639 034	NS
	rs7173301	38057135	Intron 12	G/A	0.25	0.211	0.254 551	NS
	rs12442713	38068058	Intron 15	C/A	0.294	0.244	0.227 739	NS
	rs16970132	38069064	Intron 15	G/A	0.221	0.267	0.199 312	NS
	rs2291626	38077998	Intron 19	T/C	0.265	0.167	0.048 587	NS
	rs2412462	38088462	Intron 25	G/A	0.291	0.289	0.166 460	NS
	rs4432245	38111773	Intron 36	C/T	0.481 (0.473)	0.489	$6.39\times 10^{-6}$ (0.03)	0.048
	rs711906	38112983	Intron 37	A/G	0.47 (0.471)	0.489	$4.38\times 10^{-6}$ (0.01)	0.048

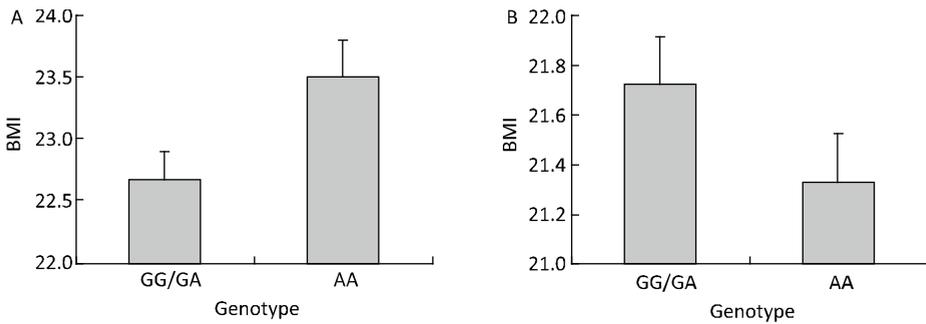
**Note.** A. The former allele represents the minor one of each locus; B. Minor allele frequency calculated in initial GWA sample; data presented in parenthesis were the MAFs calculated in replication sample; C. Minor allele frequency reported for the Chinese or Asians in the public database such as HapMap or dbSNP; D.  $P$  value for association using single-SNP test in initial GWA sample; data presented in parenthesis were the  $P$  values for association in replication sample; E. FDR  $q$  value is FDR correction value for multiple testing at the genome-wide level for initial GWA analysis; 'NS' means not significant.

For significant variants in the EIF2AK4 gene, we also observed evidence of significant marker-marker interactions between rs4432245 and rs711906 ( $P=4.76 \times 10^{-6}$ ). These interactions between markers illustrate the importance of considering them jointly in BMI genetic analysis and suggest potential patterns of biological interaction contributing to BMI variation.

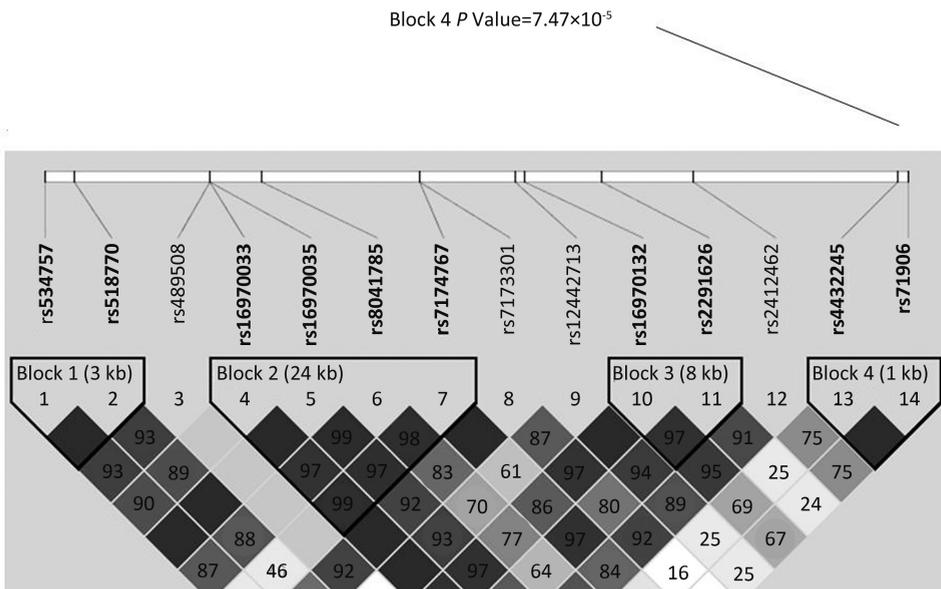
Figure 3 shows the LD pattern and haplotype block structure of the EIF2AK4 gene. In the EIF2AK4 gene blocks, the two significant SNPs (rs4432245 & rs711906) were in strong LD and formed one haplotype block. The haplotypes in this block structure were strongly associated with BMI ( $P=7.47 \times 10^{-5}$ ).

**Population Stratification**

For testing the potential population stratification of our sample, we randomly selected 1000 unlinked markers to cluster our subjects. From the triangle plot generated by STRUCTURE, all 597 subjects were tightly clustered together and could not be assigned to any subgroup. This structure analysis suggests that there is no significant population stratification in our sample. The population stratification analyses using genome control and PCA were in consistent with the results by STRUCTURE, which can be found by another published research using the same subjects in our group<sup>[38]</sup>, (no details here). All of these results indicated that potential population stratification in this homogeneous Chinese population was very minimal.



**Figure 2.** The distribution of age and sex adjusted BMI (least square means) in different genotype groups of rs711906 in EIF2AK4 gene for GWA study (A) and replication study (B). The data are presented as mean (SE).



**Figure 3.** Haplotype blocks within EIF2AK4 gene and the  $P$  value of association between block 4 and BMI.

### Comparison of our GWA with Previous GWLs and GWAs

Table 4 lists regions identified in previous linkage studies that were confirmed by strong association signals ( $P < 10^{-4}$ ) in the current GWA study<sup>[10,16-18,20,46-58]</sup>; in instances where multiple SNPs within a region were associated with BMI, we only

presented data for the SNP with the highest association signal. The strong association signals that we detected for these previously implicated linkage regions partially suggested the reasonable power and utility of our association analyses for identifying genes that influence BMI variation.

Table 5 lists several genes that were associated with BMI in previous GWA studies. Our association

**Table 4.** Genomic Regions Linked to BMI in Previous Studies, Confirmed by Results of the Current GWA Study

Genome Wide Linkage (GWL)				Genome Wide Association (GWA)			
marker <sup>B</sup>	LOD <sup>A</sup>	Population	Ref	dbSNP RS ID	Associated Gene <sup>B</sup>	P Value <sup>C</sup>	Cytoband
D4S2632	6.1						
D4S3350	9.2	utah	45	rs16883786	PCDH7	8.20×10 <sup>-5</sup>	4p15.1
D4S2912	4.5	Mexican Americans	49				
D12S2070	2.98	European American	54	rs206952	MSI1	0.0001	12q24.31
D6S287	4.06	French	18	rs11968468	PLN	0.0002	6q22.31
D17S949	3			rs3809700	ST6GALNAC2	7.22×10 <sup>-5</sup>	17q25.1
D5S433	2.28			rs10514384	-	0.0001	5q15
D10S587	2.9	French	16	rs10885378	VTI1A	2.25×10 <sup>-5</sup>	10q25.2
D19S418	3.59			rs2544785	RPL18	0.0004	19q13.32
D3S2427	3.3	White American	46	rs9833131	EPHB3	0.001	3q27.1
D3S1764	3.45	Black American		rs2369949	ACAD11	0.0003	3q22.1
D3S1259		White American		rs4680792	RBMS3	0.003	3p24.1
D7S3051				rs2711028	OSBPL3	0.0001	7p15.2
D7S2847			53	rs42172	PIK3CG	0.007	7q22.3
D14S617	>2	Japan, China		rs4344663	C14orf118	0.0003	14q24.3
GATA67G11				rs13337356	-	0.003	16q12.2
D17S947				rs2273026	SHMT1	0.002	17p11.2
D7S817	3.8	Nigeria, Yoruba	20	rs7798775	HECW1	0.0001	7p14.1
D11S2000	3.3			rs7945321	TRPC6	8.05×10 <sup>-5</sup>	11q22.1
D7S1804	3.2	White American	52	rs125095	PLXNA4B	0.001	7q32.3
D13S257	3.2			rs4942014	ELF1	0.001	13q14.11
D2S1788	3.08			rs848641	FEZ2	0.0008	2p22.2
D7S3056-D7S2477	2.53	White	51	rs10951131	CARD11	6.81×10 <sup>-5</sup>	7p22.2
D12S1052-D12S1064	3.41			rs17110690	TPH2	0.0002	12q21.1
D4S1647	2.63			rs2567397	PPP3CA	0.009	4q23
GATA8B01	2.56	African American	50	rs10958163	LOC138046	0.0003	8q21.13
D10S212	2.06			rs1917847	C10orf120	6.10×10 <sup>-5</sup>	10q26.13
D12PAH	2.6			rs7953150	PTPN11	0.003	12q24.13
D7S2557	2.9	White American	47	rs2529754	SP8	0.0005	7p15.3
D7S484	2.4			rs6463435	-	7.29×10 <sup>-5</sup>	7p12.3
D5S1505	2.2			rs17141793	-	0.0003	5q23.1
D2S347	4.04	European American	10	rs3931840	RALB	0.0005	2q14.2
D5S1725-D5S1462	2.4			rs423449	AP3B1	2.95×10 <sup>-5</sup>	5q14.1
D8S556-D8S592	2	White American	48	rs4147527	SAMD12	0.0003	8q24.12
D10S1435-D10S189	2.3-2.7			rs10904363	AKR1CL2	3.24×10 <sup>-6</sup>	10p15.1
D14S283-D14S742-D14S1280	2.2-2.4			rs1188538	OR11G2	0.0009	14q11.2
SE30	2.13			rs6917225	LYRM4	0.0002	6p25.1
D7S3056-D7S2477	2.4	Dutch	17	rs12055909	FERD3L	0.0004	7p21.1
-	2.23	Chinese	55	rs10952332	GALNTL5	6.24×10 <sup>-5</sup>	7q36.1
D13S265	2.09	Samoans	56	rs9301947	GPC6	0.001	13q31.3
AFMb035xb9	2.18	European American	57	Rs9640008	ZFAND2A	0.0009	7p22.3
AAT013	2.03				FAM44B	0.001	5q35.2

**Note.** A. Peak LOD scores >2.0 in previous GWLs. B. '-' represents not available. C. Our peak association signals in the corresponding regions identified in GWLs.

results provided supporting replication association evidence for some of these genes (e.g. INSIG2 on chromosome 2q14.1, PFKP on 10p15, FTO on 16q12, MC4R on 18q22, MRPS22 on 3q22, CDKAL1 on 6p22.3, and KLF9 on 9q21). For others, however (e.g. CTNBL1 on 20q11), the association with BMI could not be replicated in our Chinese sample.

## DISCUSSION

We identified a novel gene that might influence BMI variation in the Chinese by a powerful GWA study. In particular, the two significant SNPs, rs4432245 and rs711906, identified by the GWA study were successfully replicated by a different Chinese sample. The major lines of evidence supported the significance of the SNPs, rs4432245 and rs711906, within EIF2AK4 to BMI.

EIF2AK4 belongs to a family of kinases that phosphorylate the alpha subunit of eukaryotic translation initiation factor-2 leading to down-regulation of protein synthesis in response to a variety of cellular stresses. Our GWA study in the Chinese provided the first evidence of association between EIF2AK4 gene and obesity. Currently, this gene was not shown to have any function directly relevant to obesity in humans. Guo et al. have shown that in knock-out mice study, there is no significant body weight difference against wild-type mice under regular chow. However, during leucine deprivation in

mice, significant differences of adiposity was shown as EIF2AK4 down-regulated genes and enzyme activity related to triglyceride synthesis<sup>[59]</sup>. Through regulating genes related to the synthesis of fatty acids, EIF2AK4 has a profound effect on the synthesis and storage of triglycerides and overall energy homeostasis. So it is speculated that EIF2AK4 may act as a master regulator of metabolic adaptation to nutrient deprivation, resulting in the process of fat accumulation. These biological evidences, together with the significant associations found in our initial GWAS and in our replication study, strongly support EIF2AK4 as a novel candidate gene influencing the variation of BMI. Functional analysis by FASTSNP suggested that rs4432245 and rs711906 might serve as binding sites for intronic enhancers in the EIF2AK4 gene. A 'C → T' change at rs4432245 may potentially delete one binding site for transcription factor MZF1, whereas an 'A → G' change at rs711906 may delete binding sites for three distinct transcription factors (CdxA, S8, and Nkx-2).

The opposite effect of allele 'A' of rs711906 on BMI variation may be caused by several factors. First, the direction of allelic association may flip when the target risk allele is inversely correlated with another risk allele at another locus, or positively associated with a protective allele at another locus. And the flip-flop associations depend on allele frequency and inter-locus correlation (Abstract of the presentation at 11th International Congress of Human Genetics, 2006, Australia). In our study, the unknown inverse

**Table 5.** Comparison of Current Study Results with Previously Published Original GWA Studies for BMI

Results of the Published Original GWA Studies <sup>A</sup>					Results of Current GWA Study <sup>B</sup>			
SNP	Associated Gene	Cytoband	<i>p</i> Value	Ref.	SNP	Associated Gene	Cytoband	<i>P</i> Value
rs7566605	INSIG2	2q14.1	0.0026	33	rs1866407	INSIG2	2q14.1	0.002
rs17782313	MC4R	18q22	2.8 x10 <sup>-15</sup>	35	rs1943229	MC4R	18q22	0.005
rs7638110	MRPS22	3q23	4.6x10 <sup>-8</sup>	34	rs10460842	MRPS22	3q23	6.9 x10 <sup>-5</sup>
rs6013029	CTNBL1	20q11.23-q12	2.7 x10 <sup>-7</sup>	30	rs4811211	CTNBL1	20q11.23-q12	0.08
rs9930506	FTO	16q12.2	8.6 x10 <sup>-7</sup>	32	rs13337356	FTO	16q12.2	0.003
rs9939609	FTO	16q12.2	3 x10 <sup>-10</sup>	31				
rs9939609	FTO	16q12.2	1.5 x10 <sup>-7</sup>	36				
rs6602024	PFKP	10p15	4.9 x10 <sup>-6</sup>	32	rs2388384	PFKP	10p15	0.001
rs2206734	CDKAL1	6p22.3	1.4 x 10 <sup>-11</sup>	37	rs9350257	CDKAL1	6p22.3	0.001
rs11142387	KLF9	9q21	1.3 x 10 <sup>-9</sup>	37	rs6560130	KLF9	9q21	0.01

**Note.** A. Only studies conducted for BMI on population-based samples were included. B. The most significant SNP in current GWA study within the gene identified by published GWA studies.

correlation of rs711906 with other risk locus may exist. Czarnomska et al. have also shown that a set of genes control the impact of the *Apc*<sup>Min</sup> mutation in both organs but with opposite effects<sup>[60]</sup>. Second, some studies have shown that environmental covariates will influence the effect direction of gene variants. Eder et al. discovered opposite effects of CD14/-260 on serum IgE levels in children raised in different environments<sup>[61]</sup>. Reneland et al. found that rs1498608 in PDE4D gene showed an opposite relationship with BMD variation, indicating that the variant's effect may be context-dependent<sup>[62]</sup>. As our replication sample comes from south China, which is significantly different from the initial GWA study sample recruited in north China, the different environment, like living and dietary habit may influence the effect direction of the allele. However, it needs to be confirmed in other independent samples.

The most significant association of rs4633 was not successfully confirmed in the replication study. This may be explained in two aspects. First, it was just a false-positive signal in the GWA study. Second, the two samples for initial GWA study and follow-up replication study differed largely by age. Tworoger et al. found that postmenopausal women exercisers with the COMT Val/Val genotype had a smaller decrease in BMI than women with neither allele (-1.0 vs. +0.1 kg/m<sup>2</sup>,  $P=0.009$ )<sup>[63]</sup>. The genetic variability of COMT gene can affect estrogen and androgen<sup>[64]</sup>. As the old people suffered an obvious loss of estrogen and androgen, while the young could maintain these hormones at a steady level, we may guess that this gene's effect on fat regulation through estrogen and androgen could only be observed in the old people. However, to ascertain this assumption further in depth investigations will be needed.

The prevalence of obesity has been shown to vary widely across different ethnics/populations<sup>[65-66]</sup>, and ethnic disparity in the genetic background has been considered as an important contributing factor that helps explain this variation. Henderson et al. found a statistically significant interaction between race/ethnicity and obesity status ( $P=0.005$ ) in a multivariate regression of IGF-I levels<sup>[67]</sup>. Li et al. showed that variants in FTO gene, which was significantly and consistently associated with BMI in populations of European origin, were not associated with BMI in a Chinese Han population<sup>[68]</sup>. Similarly, the association of the CTNBL1 gene with BMI identified by our research group in Caucasian subjects<sup>[30]</sup> could not be replicated in the present

study in Chinese. Collectively, these data support the concept that the genetic determinants for BMI or obesity related phenotypes may partially vary across different ethnic groups.

Using the software Genetic Power Calculator<sup>[69]</sup>, the estimated power of the present GWA sample to detect a gene accounting for 1.5% of BMI variation is 86.4% at a threshold  $P<0.05$  (used in the replication study). We can confirm some common genes important for both ethnic populations, but the present study suggests that the EIF2AK4 gene may potentially be an ethnic-specific gene regulating BMI variation in the Chinese. First, the MAFs of the two significant SNPs in the EIF2AK4 gene are distinctly different between the Chinese and the Caucasians, (0.49 and 0.05, respectively), according to the dbSNP database. Second, in the Chinese we found a significant association between the EIF2AK4 gene and BMI in our initial GWA and follow-up replication studies; we did not, however, find a significant association between EIF2AK4 and BMI in our GWA study of 1000 Caucasians (data not shown).

In summary, our GWA study has identified a novel candidate gene, EIF2AK4, that is significantly associated with BMI variation in Chinese. The association of the EIF2AK4 gene is suggested to be 'ethnic specific' in Chinese. Follow-up studies could be pursued by replicating in other larger samples and populations to validate the specific associations, genotyping denser SNPs or re-sequencing the novel genomic region containing the gene to identify the causal variants and performing molecular functional studies to define the exact roles that the gene plays in regulating fat metabolism.

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