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Original Article

Association Study of Three Gene Polymorphisms Recently Identified by a Genome-Wide Association Study with Obesity-Related Phenotypes in Chinese Children

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Abstract
Objective: This study aimed to examine associations of three single-nucleotide polymorphisms (SNPs) with obesity-related phenotypes in Chinese children. These SNPs were identified by a recent genome-wide association (GWA) study among European children. Given that varied genetic backgrounds across different ethnicity may result in different association, it is necessary to study these associations in a different ethnic population. ***Methods:*** A total of 3,922 children, including 2,191 normal-weight, 873 overweight and 858 obese children, from three independent studies were included in the study. Logistic and linear regressions were performed, and meta-analyses were conducted to assess the associations between the SNPs and obesity-related phenotypes. ***Results:*** The pooled odds ratios of the A-allele of rs564343 in *PACS1* for obesity and severe obesity were 1.180 ($p = 0.03$) and 1.312 ($p = 0.004$), respectively. We also found that rs564343 was nominally associated with BMI, BMI standard deviation score (BMI-SDS), waist circumference, and waist-to-height ratio ($p < 0.05$). ***Conclusions:*** We showed for the first time that the rs564343 in *PACS1* was associated with risk of severe obesity in a non-European population. This SNP was also found to be associated with common obesity and various obesity-related phenotypes in Chinese children, which had not been reported in the original study. The results demonstrated the value of conducting genetic researches in populations with different ethnicity.

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Introduction

It is widely acknowledged that obesity is an increasingly important health problem in both developed and developing countries. A systematic analysis recently reported that, the global prevalence of overweight and obesity has risen by 27.5% for adults and 47.1% for children over the past three decades [1]. In the US, more than two-thirds (68.5%) of adults and nearly one-third (31.8%) of youth were either overweight or obese in 2011–2012 [2]. In China, the prevalence of overweight and obesity among urban boys and girls were 28.14% and 15.56%, and that among rural boys and girls were 18.62% and 11.81% in 2010, showing an increase compared with the data of 2005 [3]. Obesity is an important risk factor of many diseases, including type 2 diabetes mellitus, hyperlipidemia, hypertension, cardiovascular disease, and cancers [4, 5].

Genetic factors play an indispensable role in the individual's predisposition to obesity [6–8]. The heritability of the variance ofBMI ranged from 30% to 70% [9], and it is estimated that heritability for BMI or obesity is higher in children than in adults [10]. In recent years, genome-wide association (GWA) studies have provided evidence for genetic risk loci for obesity [11–14]. Many replication studies for these loci have been conducted in multiple ethnic populations, including Chinese [15–20]. Two recently published studies by Wang et al. [19] and Meng et al. [20] from our research group confirmed some loci identified by GWA studies to be associated with obesity in Chinese children, and identified ethnic difference of effect size for some loci (supplemental table 1, available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=471487*).

A recent GWA study identified four new loci (rs564343 in phosphofurin acidic cluster sorting protein 1 (*PACS1*), rs11109072 in rhabdomyosarcoma 2 associated transcript (*RMST*), rs1957894 in protein kinase C eta (*PRKCH*) and rs11208659 in leptin receptor (*LEPR*)), which were associated with severe early-onset obesity among European children [21]. To our knowledge, these new loci have not been studied in independent populations with different ethnicity. Given that varied genetic backgrounds across different ethnicity may result in different association, it is necessary to study these associations in different ethnic populations. Therefore, we conducted the present study for 3 loci (*PACS1, RMST, PRKCH*) with the minor allele frequency ≥0.20 based on our sample size to explore whether these loci were associated with obesity in Chinese children.

Subjects and Methods

Subjects

We conducted three independent studies with a total of 3,922 subjects aged 6–18 years, recruited from the urban regions of Beijing, China. The first study on adolescent lipids, insulin resistance, and candidate genes (ALIR) involved 937 participants from 9 middle schools of the Dongcheng District of Beijing. The second study was from the Comprehensive Prevention Project for Overweight and Obese Adolescents (CPOOA) with physical exercise and healthy nutrition as instruments, which was conducted in 3 middle schools and 2 elementary schools of the Haidian District of Beijing, involving 1,093 participants. Both studies are case-control studies, and the ascertainment strategies and other detailed information have been previously described [22, 23].

The third study was a School-Based Comprehensive Intervention on Childhood Obesity (SCICO), performed in 8 elementary schools of the Haidian District of Beijing, recruiting 2,125 subjects [24, 25]. The children who were underweight according to the Chinese national screening criteria for malnutrition of children aged 6–18 years [26] and those without collected blood samples were excluded. Finally, a total of 1,892 individuals of the third study were involved in this study, including 1,584 normal-weight children (834 males, mean age 8.72 ± 1.60 years, mean BMI 15.94 ± 1.42 kg/m^2), 155 overweight children (98 males, mean age 8.87 ± 1.60 years, mean BMI 19.82 ± 1.60 kg/m^2) and 153 obese children (98 males, mean age 8.82 ± 1.45

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years, mean BMI 23.77 ± 2.77 kg/m^2). Based on the uniform BMI percentile criteria for screening overweight and obesity of children aged 7–18 years (supplemental table 2, available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=471487*), which were determined in a representative Chinese children sample [27], the participants were defined as obese (age- and gender-specific BMI ≥ 95th percentile) or overweight (95th ≥ age- and gender-specific BMI ≥ 85th percentile). After exclusion of underweight children, those with an age- and gender-specific BMI less than the 85th percentiles are considered as normal-weight students. For children aged 6 years, the criteria developed by Li et al. [28] were used (supplemental table 2, available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=471487*). The BMIs of the severely obese groups were above the 97th percentile of a representative Chinese population [29]. Those with any cardiovascular disorder or metabolic disease were excluded.

Written informed consent was provided by all participants and their parents, in the case of minors. The first and second studies were approved by the Ethic Committee of Peking University Health Science Center, while the third study was approved by the Ethic Committee of Chinese Center for Disease Control and Prevention.

Anthropometric, Biochemical and Blood Pressure Measurements

All measurements in the three studies were performed with the same methods. Anthropometric measurements, including height, weight, waist and hip circumferences, were conducted according to standard protocols [30]. BMI (kg/m^2) and the sex-and age-specific BMI standard deviation score (BMI-SDS) were calculated. The latter was calculated according to the growth reference data of the World Health Organization for children aged 5–19 years [31]. Fasting venous blood samples were taken for measurement of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and plasma glucose using the biochemical autoanalyzer (ALIR and CPOOA: Hitachi7060, Tokyo, Japan; SCICO: Olympus AU400, Tokyo, Japan). Blood pressure was measured using an auscultation mercury sphygmomanometer with an appropriate cuff size for children in these studies, according to the recommendation of the National High Blood Pressure Education Program Working Group in Children and Adolescents [32]. Mean systolic and diastolic blood pressures (SBP and DBP) were calculated by averaging three measurements.

SNPs Selection and Genotyping

Given the sample size (2,191 normal-weight children and 858 obese children), and the assumed effect size of 1.2 for an allele frequency ≥ 0.20, the power for detecting positive association was estimated to be 75%. So we selected three SNPs (rs564343, rs11109072 and rs1957894) with the minor allele frequency ≥ 0.20 in the Chinese population (Hapmap database: *http://hapmap.ncbi.nlm.nih.gov*), excluding rs11208659 in *LEPR* (minor allele frequency = 0.07). Multiplex SNP assays designs failed for one SNP (rs1957894 in *PRKCH*), so we replaced it with one proxy SNP (rs1957893) having strong linkage disequilibrium (r^2 = 1.00) in Chinese population in HapMap Data Release 24/Phase II Nov08 (*http://hapmap.ncbi.nlm.nih.gov*).

The genomic DNAs of subjects were isolated from blood leukocytes of fasting venous blood samples with the phenol-chloroform extraction method. Genotyping of the three SNPs were carried out with the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS, Sequenom). The detailed genotyping process has been described previously [19, 20]. The call rates of the three SNPs were more than 99.9%.

Statistical Analyses

The differences in general characteristics among three groups were tested with one-way analysis of variance (*ANOVA*) (continuous variables) or χ^2 test (categorical variables). Hardy-Weinberg equilibrium (HWE) was tested with χ^2 statistics in the normal-weight group. *F*-statistics (F_{ST}) was calculated, as a metric estimating the effect of population subdivision, according to the following formula: $F_{ST} = (P_1 - P_2)^2 / ((P_1 + P_2) \times (2 - (P_1 + P_2)))$, where P_1 is allele frequency in the original study population and P_2 is allele frequency in the present study population [33, 34]. An F_{ST} value between 0 and 0.05 suggests little genetic differentiation; a value between 0.05 and 0.15 moderate differentiation, a value between 0.15 and 0.25 great differentiation, and values above 0.25 very great differentiation [35].

Multivariate logistic regression model was performed to evaluate the independent effect of each SNP on the risk of obesity, severe obesity or overweight. Linear regression analysis was performed to evaluate the independent effect of each SNP on other obesity-related phenotypes. All analyses used the additive model with adjustment for sex, age, and age square, with or without BMI. For the proxy SNPs (rs1957893 in *PRKCH*), the allele which was correlated with the effect allele of the original SNP in the original study was defined as the effect allele, in the convenience of comparing our results with the published data [21].

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A two-sided p value < 0.05 was considered as nominally significant. p < 0.001 (i.e., 0.05 / (3 SNPs × (4 categorical variables + 13 continuous variables)) = 0.05 / 51 = 0.001) was considered as statistically significant after multiple testing using Bonferroni correction. Statistical analyses were performed using SPSS 18.0 software (IbM Corp., Armonk, NY, USA). Power calculations were performed with the method for genetic study of case-control (unmatched) by Quanto software (University of Southern California, Los Angeles, CA, USA).

Meta-Analysis

To calculate the pooled estimates of the association between each SNP and obesity, severe obesity, overweight or other obesity-related phenotypes, we conduct meta-analysis with the Inverse Variance method. Odds ratio (OR) was used as the effect size of categorical variables, while mean difference (MD) was used as the effect size of continuous variables. Heterogeneity among studies was assessed by the heterogeneity *Q* statistic. A p value less than 0.05 on the *Q* test indicated heterogeneity among the studies. The I^2 statistic, which is a quantitative measure of inconsistency across studies, was also calculated, with >70%, indicating high heterogeneity [36]. When there was little heterogeneity among three studies, a fixed-effect model was used. Otherwise, a random-effect model was used [37]. Revman 5.2 (available at the website of Cochrane Collaboration: *www.cc-ims.net/ revman*) was used for the meta-analysis.

Results

The general characteristics of the study groups were presented in table 1. Except for age in the SCICO study (p = 0.442), sex and age were significantly different among the normal-weight, overweight and obese groups in the three independent studies (p < 0.05). Obesity-related phenotypes (BMI, BMI-SDS, waist and hip circumferences, waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), total cholesterol, triglyceride, LDL-C, fasting plasma glucose (FPG), SBP, and DBP) were the highest in obese groups among the three studies, except for HDL-C.

Table 2 showed the genotypes and allele frequencies of the 3 SNPs. The effect allele frequencies of rs564343 and rs1957893 in our study were similar to those reported in the HapMap Han Chinese, and that of rs11109072 was similar to that among Chinese and Japanese individuals (CHB+JPT) in the HapMap database (*http://hapmap.ncbi.nlm.nih.gov/*). All effect allele frequencies in our study were higher than those of the original study. Based on the F_{ST} values, we found moderate differentiations for the three SNPs between our study and the original study (0.05 < F_{ST} value < 0.15, table 2). In three different studies, the genotypes of 2 SNPs (rs564343 and rs11109072) were in HWE among the normal-weight children (p > 0.05). For rs1957893 in *PRKCH,* we observed no evidence for deviations from HWE in the first and second studies (p = 0.907 and 0.111, respectively), while in the third study we observed a deviation from HWE with marginal significance (p = 0.028) (table 2), but a double-checking of the genotype data revealed no obvious genotyping errors.

The effect estimates of the 3 SNPs on obesity-related phenotypes in 3,922 individuals of the three studies were pooled for meta-analysis using fixed effects model. The pooled OR of the A-allele of rs564343 for severe obesity was 1.312 (95% confidence interval (CI) 1.090–1.578, p = 0.004, fig. 1), indicating that each A-allele is associated with a 31.2% increased risk for severe obesity. However, the association was not significant after multiple correction. The pooled OR of the A-allele of rs564343 in *PACS1* for obesity was 1.180 (95% CI 1.013–1.375, p = 0.03, fig. 2), indicating that each A-allele is associated with a 18.0% increased risk for obesity. We also found significant association of rs564343 with risk of overweight and obesity combined (OR = 1.144, 95% CI 1.011–1.294, p = 0.03; supplemental fig. 1, available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=471487*), and directionally consistent associations of rs564343 with risk of overweight (OR = 1.108, 95% CI 0.955–1.286, p = 0.18;

Table 1. General characteristics of the study groups *

	ALIR Study				CPOOA Study				SCICO Study			
	normal-weight	overweight	obese	p value	normal-weight	overweight	obese	p value	normal-weight	overweight	obese	p value
Number	151	400	386	–	456	318	319	–	1584	155	153	–
Male, %	88 (58.3)	250 (62.5)	269 (69.7)	0.020	196 (43.0)	200 (62.9)	215 (67.4)	<0.001	834 (52.7)	98 (63.2)	98 (64.1)	0.002
Age, years	14.81 ± 0.75	14.63 ± 0.56	14.60 ± 0.55	0.001	11.80 ± 3.15	11.54 ± 2.63	10.73 ± 2.51	<0.001	8.72 ± 1.60	8.87 ± 1.60	8.82 ± 1.45	0.442
BMI, kg/m^2	20.41 ± 1.83	25.08 ± 1.00	30.02 ± 3.07	<0.001	18.24 ± 2.46	22.33 ± 2.34	25.82 ± 3.64	<0.001	15.94 ± 1.42	19.82 ± 1.60	23.77 ± 2.77	<0.001
BMI-SDS	0.16 ± 0.67	1.59 ± 0.24	2.47 ± 0.44	<0.001	-0.05 ± 0.72	1.55 ± 0.30	2.52 ± 0.53	<0.001	–0.26 ± 0.70	1.50 ± 0.29	2.59 ± 0.62	<0.001
Waist circumference, cm	69.40 ± 6.55	80.87 ± 4.78	93.05 ± 9.15	<0.001	63.15 ± 7.45	73.98 ± 7.90	82.20 ± 10.73	<0.001	55.01 ± 5.03	66.58 ± 7.41	76.45 ± 8.83	<0.001
Hip circumference, cm	89.99 ± 4.93	98.98 ± 3.99	107.18 ± 6.18	<0.001	80.17 ± 10.61	87.75 ± 9.67	92.84 ± 10.76	<0.001	–	–	–	–
WHR	0.77 ± 0.06	0.82 ± 0.05	0.87 ± 0.06	<0.001	0.79 ± 0.05	0.85 ± 0.05	0.89 ± 0.06	<0.001	–	–	–	–
WHtR	0.42 ± 0.04	0.48 ± 0.02	0.55 ± 0.05	<0.001	0.41 ± 0.03	0.48 ± 0.03	0.54 ± 0.05	<0.001	0.41 ± 0.03	0.48 ± 0.03	0.55 ± 0.05	<0.001
Total cholesterol, mmol/l	4.26 ± 0.67	4.48 ± 0.82	4.64 ± 0.80	<0.001	4.09 ± 0.68	4.06 ± 0.67	4.19 ± 0.68	0.038	3.91 ± 0.62	4.04 ± 0.59	4.11 ± 0.67	<0.001
Triglyceride, mmol/l	0.73 ± 0.26	0.88 ± 0.39	1.22 ± 0.74	<0.001	0.80 ± 0.31	0.89 ± 0.35	1.10 ± 0.51	<0.001	0.73 ± 0.34	0.88 ± 0.37	1.11 ± 0.68	<0.001
LDL-C, mmol/l	2.14 ± 0.65	2.49 ± 0.76	2.84 ± 0.83	<0.001	2.08 ± 0.53	2.23 ± 0.54	2.40 ± 0.56	<0.001	1.66 ± 0.41	1.81 ± 0.42	1.90 ± 0.47	<0.001
HDL-C, mmol/l	0.81 ± 0.20	0.87 ± 0.21	0.86 ± 0.20	0.006	1.64 ± 0.32	1.47 ± 0.27	1.38 ± 0.27	<0.001	1.41 ± 0.27	1.35 ± 0.29	1.26 ± 0.26	<0.001
FPG, mmol/l	4.44 ± 0.43	4.39 ± 0.63	4.45 ± 0.65	0.351	5.26 ± 0.38	5.40 ± 0.40	5.45 ± 0.40	<0.001	4.32 ± 0.62	4.39 ± 0.63	4.39 ± 0.60	0.176
SBP, mm Hg	111.37 ± 9.84	120.37 ± 11.56	128.79 ± 14.23	<0.001	101.06 ± 13.19	106.88 ± 13.65	111.70 ± 15.35	<0.001	101.71 ± 9.03	104.97 ± 8.92	108.40 ± 9.39	<0.001
DBP, mm Hg	70.93 ± 7.29	75.49 ± 7.37	79.13 ± 8.20	<0.001	50.91 ± 11.83	52.57 ± 11.49	53.38 ± 12.77	0.016	66.35 ± 7.91	68.99 ± 8.14	69.03 ± 7.65	<0.001

ALIR = Adolescent Lipids, Insulin Resistance and Candidate Genes; BMI-SDS = BMI standard deviation score; CPOOA = Comprehensive Prevention Project for Overweight and Obese Adolescents; DBP = diastolic blood pressure; FPG = fasting plasma glucose; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SCICO = School-Based Comprehensive Intervention on Childhood Obesity; SBP = systolic blood pressure; SNP = single nucleotide polymorphism; WHR = waist-to-hip ratio; WHtR =waist-to-height ratio.

*Data are expressed as mean ± standard deviation, if not indicated otherwise, – not available.

Table 2. Genotyping information of three SNPs in Chinese children

SNP	Nearest gene	Chr.	Position	Allele (1/2)[a]	EAF[b]	EAF[c]	EAF[d]	Call rate, %	F_{ST}	ALIR Study		CPOOA Study		SCICO Study	
										genotype [e]	HWE p value[f]	genotype	HWE p value	genotype	HWE p value
rs564343	*PACS1*	11	65651742	A/G	0.72	0.69	0.41	99.9	0.096	72/61/18	0.366	233/178/44	0.246	809/640/132	0.732
rs11109072	*RMST*	12	96425401	A/C	0.26	0.23	0.03	99.9	0.107	9/62/80	0.505	32/172/251	0.733	105/635/843	0.319
rs1957893	*PRKCH*	14	60977864	T/C	0.33	0.29	0.08	99.9	0.115	20/69/62	0.907	58/187/210	0.111	185/654/742	0.028

ALIR = Adolescent Lipids, Insulin Resistance and Candidate Genes; Chr. = chromosome; EAF = effect allele frequency; CPOOA = Comprehensive Prevention Project for Overweight and Obese Adolescents; Position = NCBI build 36.3 (NCBI, Bethesda, MD); SCICO = School-Based Comprehensive Intervention on Childhood Obesity; SNP = single nucleotide polymorphism.

[a]1 = effect allele, 2 = other allele.

[b]Effect allele frequency in all genotyped individuals of the present study.

[c]Effect allele frequency reported in the HapMap Han Chinese, and that of rs11109072 is from HapMap database among Chinese and Japanese individuals (CHB+JPT).

[d]Effect allele frequency of SNPs in the original study, and that of the proxy SNP (rs1957893) is from NCBI database among the same populations that in the discovery study.

[e]Genotypes of normal weight group (11/12/22).

[f]HWE p value in normal-weight Chinese children.

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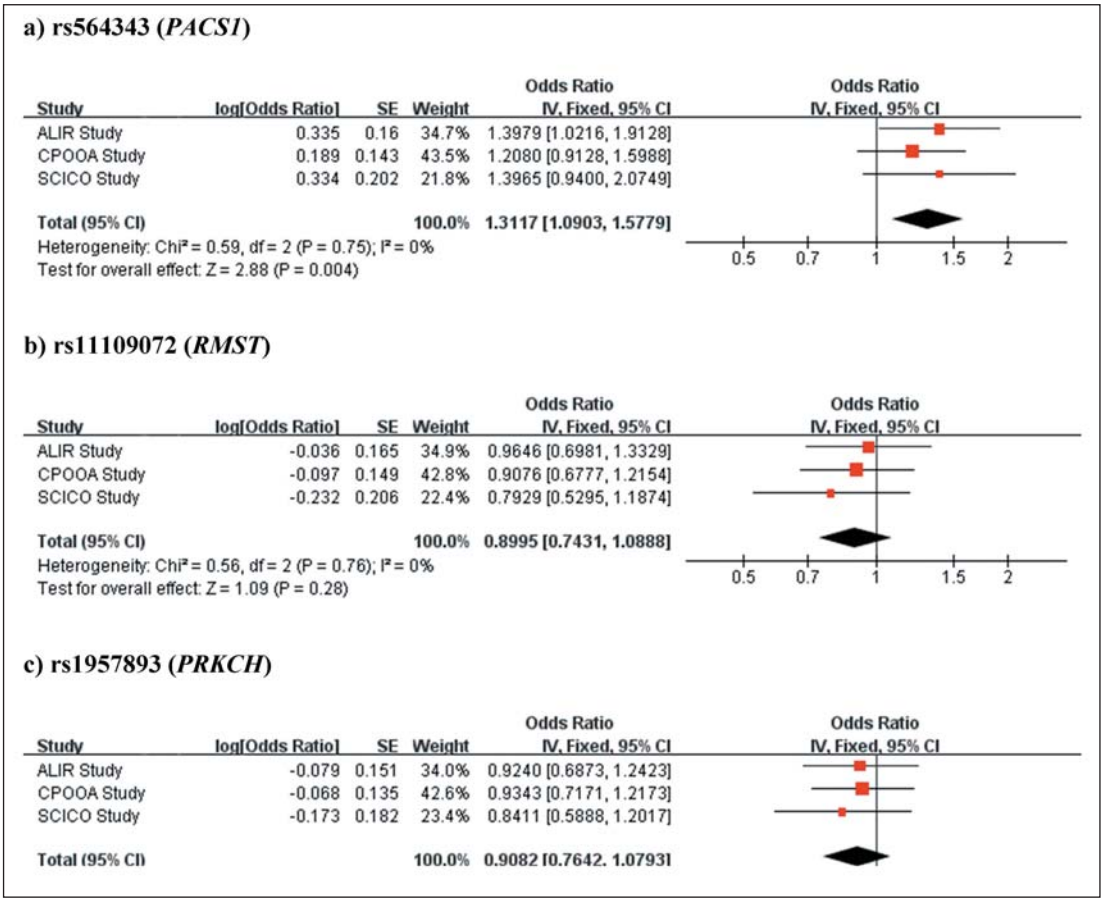

Fig. 1. Odds ratios with the 95% CI for the association between **a** rs564343 in *PACS1*, **b** rs11109072 in *RMST*, **c** rs1957893 in *PRKCH* and severe obesity in the meta-analysis.

supplemental fig. 2, available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=471487*).

In further analyses, we also found the A-allele of rs564343 was associated with BMI, BMI-SDS, waist circumference, and WHtR after adjusting for sex, age, and age square under the additive model (table 3). Each A-allele of rs564343 was associated with an increase of 0.167 kg/m^2 in BMI (p = 0.03), 0.058 in BMI-SDS (p = 0.02), 0.443 cm in waist circumference (p = 0.04), and 0.003 in WHtR, but none was significant after Bonferroni correction. Additional adjustment for BMI showed the association of rs564343 with waist circumference and WHtR was abolished.

No significant association was observed between the other two SNPs and risk of obesity or other obesity-related phenotypes (fig. 1–2; supplemental tables 3–4, available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=471487*).

Discussion

To our knowledge, this is the first study to explore the association of the three SNPs, which were identified by a recent GWA study by Wheeler et al. [21] in European children, with obesity-related phenotypes in another population with different ethnicity, i.e., in Chinese

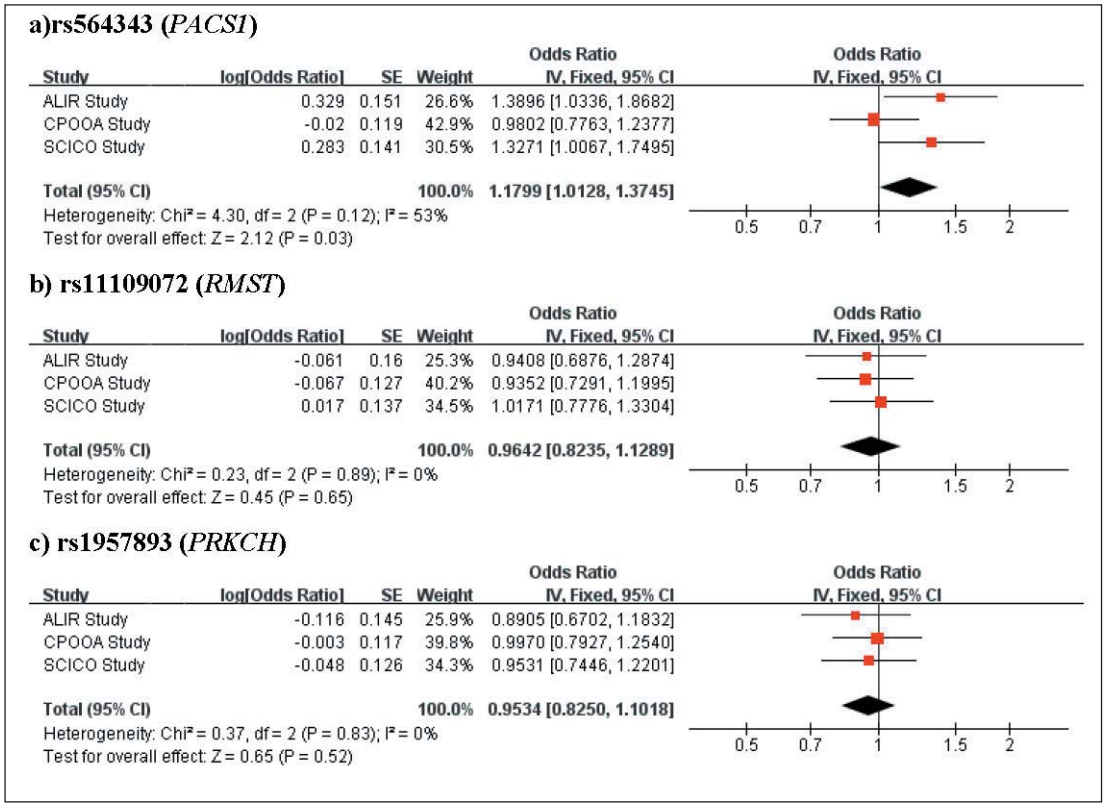

Fig. 2. Odds ratios with the 95% CI for the association between **a** rs564343 in *PACS1*, **b** rs11109072 in *RMST*, **c** rs1957893 in *PRKCH* and obesity in the meta-analysis.

children. The rs564343 (*PACS1*) was found to be associated with both severe obesity and common obesity. Besides, rs564343 polymorphism was also observed to be nominally associated with several obesity-related phenotypes (BMI, BMI-SDS, waist circumference, and WHtR).

PACS1 is a trans-Golgi-membrane traffic regulator, directing protein cargo and several viral envelope proteins [38]. Wan et al. [39] first reported the role of PACS1, one member of a novel gene family of cytosolic sorting proteins, in the localization of trans-Golgi network (TGN) membrane proteins. PACS1 binds to the protease's phosphorylated cytosolic domain of furin, then directs its TGN localization [39]. The A-allele of rs564343 *PACS1* gene was first reported to be associated with severe early-onset obesity in the original GWA study [21]. However, the mechanism how the *PACS1* gene is involved in the pathogenesis of obesity hitherto has not been elucidated.

In the present study, we demonstrated that rs564343 in *PACS1* was robustly associated with obesity and obesity-related phenotypes in Chinese children. The consistency with the original GWA study suggested that the origin of rs564343 in *PACS1* might be relatively ancient so that the Chinese population with different ethnicity and environmental backgrounds were affected by the SNP in the same direction. Furthermore, we found that A-allele of rs564343 was associated with common obesity, as well as combined overweight and obesity, which had not been observed in the original study. These findings indicate possible ethnic differences for effect sizes of the genetic polymorphisms on obesity, which awaits more large-scaled studies in other populations.

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Table 3. The meta-analysis results of association between rs564343 in *PACS1* and various obesity-related phenotypes in Chinese children

	Model 1[a]		Model 2[b]	
	β (95% CI)	p value	β (95% CI)	p value
BMI, kg/m^2	0.167 (0.015, 0.318)	0.03*	–	–
BMI-SDS	0.058 (0.008, 0.108)	0.02*	–	–
Waist circumference, cm	0.443 (0.018, 0.868)	0.04*	0.015 (–0.149, 0.178)	0.86
Hip circumference, cm[c]	0.326 (–0.205, 0.856)	0.23	–0.130 (–0.361, 0.100)	0.27
WHR[c]	0.003 (–0.001, 0.007)	0.13	0.001 (–0.002, 0.004)	0.64
WHtR	0.003 (0.000, 0.005)	0.04*	0.000 (–0.001, 0.001)	0.92
Total cholesterol, mmol/l	–0.006 (–0.039, 0.027)	0.73	–0.010 (–0.042, 0.023)	0.57
Triglyceride, mmol/l	0.002 (–0.019, 0.022)	0.87	–0.006 (–0.026, 0.014)	0.55
LDL-C, mmol/l	–0.007 (–0.031, 0.017)	0.57	–0.012 (–0.036, 0.012)	0.33
HDL-C, mmol/l	–0.002 (–0.015, 0.010)	0.73	–0.000 (–0.012, 0.012)	0.97
FPG, mmol/l	0.012 (–0.013, 0.037)	0.33	0.012 (–0.013, 0.037)	0.36
SBP, mm Hg	0.086 (–0.424, 0.595)	0.74	–0.154 (–0.626, 0.319)	0.52
DBP, mm Hg	–0.258 (–0.677, 0.161)	0.23	–0.386 (–0.791, 0.019)	0.06

BMI-SDS = BMI standard deviation score; DBP = diastolic blood pressure; FPG = fasting plasma glucose; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SBP = systolic blood pressure; SNP = single nucleotide polymorphism; WHR = waist-to-hip ratio; WHtR = waist-to-height ratio.

[a]Model 1 was adjusted for sex, age and age square under the additive model.

[b]Model 2 was adjusted for sex, age, age square and BMI under the additive model.

[c]Data were available from the ALIR and CPOOA study (N = 2030).

*Two-sided $p < 0.05$ (nominally significant).

In addition, we extended our analyses to various obesity-related phenotypes, including anthropometric measurements (BMI, BMI-SDS, waist and hip circumferences, WHR, WHtR), serum lipids (total cholesterol, triglyceride, LDL-C and HDL-C), and other metabolic phenotypes (FPG, SBP, and DBP). We found nominal associations between rs564343 (*PACS1*) and BMI, BMI-SDS, waist circumference, and WHtR, which further supports that the association between rs564343 and obesity was robust. However, the association between rs564343 and waist circumference or WHtR did not exist after additional adjustment of BMI, suggesting that these associations were mediated by BMI.

Although the other two SNPs (rs11109072 and rs1957894) were reported to be associated with severe early-onset obesity [21], we did not observe their effects on risk of obesity or obesity-related phenotypes in the present study. There might be two reasons. First, there is ethnic differentiation between Chinese ancestry and European ancestry. The effect allele frequencies of the two SNPs in our study were similar to those reported in HapMap Han Chinese,but different from the frequencies of the original study. And the F_{ST} values of both rs11109072 and rs1957893 were between 0.05 and 0.15, indicating moderate differentiation between Chinese and Europeans. Secondly, the limited sample size and statistic power might also account for the failure in replication.

The strength of our study is the first study to explore the relationship between the three SNPs (rs564343, rs11109072, and rs1957893) and childhood obesity in another population different from the original study. The association between rs564343 in *PACS1* and obesity was found in Chinese children. Although the association of these SNPs with obesity has been established by a GWA study, information regarding the contribution of each SNP on different obesity-related phenotypes is lacking. The present study extended our analyses to various

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obesity-related phenotypes and provided more information. In addition, the participants in our studies were children aged 6–18 years. Compared with adults, it is reported that children have higher obesity heritability and usually have simple obesity without complications [12], which help to detect the effects of common variants on obesity.

However, there are a few limitations of our study. Firstly, we selected only three novel SNPs which were recently identified by a GWA study [21], but did not include rs11208659 in *LEPR* with the minor allele frequency < 0.20. More large-scaled studies involving more SNPs with lower allele frequencies are needed. Secondly, the study has limited sample size and statistical power. The true genetic effects in Chinese may be smaller than that in original population (European ancestry), described as the 'winner's curse' [40].

Conclusions

In conclusion, we for the first time showed that rs564343 in *PACS1* was associated with risk of severe obesity in non-European population. And we found that this SNP was also associated with common obesity and various obesity-related phenotypes in Chinese children, which had not been found in the original study. Our results demonstrated the value of conducting genetic studies in populations with different ethnicity and provided evidences for genetic factors associated with pediatric obesity.

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Disclosure Statement

The authors declare that they have no conflict of interest.

References

1 Ng M, Fleming T, Robinson M, et al: Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013:a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014;384:766–781.
2 Ogden CL, Carroll MD, Kit BK, Flegal KM: Prevalence of childhood and adult obesity in the United States, 2011–2012. JAMA 2014;311:806–814.
3 Ministry of Education of the People's Republic of China: Report on the physical fitness and health surveillance of Chinese school students (2010). Chi J School Health 2011;32:1024–1026.
4 Haslam DW, James WPT: Obesity. Lancet 2005;366:1197–1209.
5 Lean ME: Pathophysiology of obesity. The Proc Nutr Soc 2000;59:331–336.
6 Mayer J: Genetic factors in human obesity. Ann Ny Acad Sci 1965;131:412–421.
7 Li S, Zhao JH, Luan J, Luben RN, Rodwell SA, Khaw KT, Ong KK, Wareham NJ, Loos RJ: Cumulative effects and predictive value of common obesity-susceptibility variants identified by genome-wide association studies. Am J Clin Nutr 2010;91:184–190.
8 Chesi A, Grant SF: The genetics of pediatric obesity. Trends Endocrin Met 2015;26:711–721.
9 Herbert A, Gerry NP, Mcqueen MB, Heid IM, Pfeufer A, Illig T, Wichmann HE, Meitinger T, Hunter D, Hu FB: A common genetic variant is associated with adult and childhood obesity. Science 2006;312:279–283.
10 Min J, Chiu DT, Wang Y: Variation in the heritability of body mass index based on diverse twin studies: a systematic review. Obes Rev 2013;14:871–882.

Obes Facts 2017;10:179–190
DOI: 10.1159/000471487

11 Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, Lango AH, Lindgren CM, Luan J, Mägi R: Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet 2010;42:937–948.
12 Bradfield JP, Taal HR, Timpson NJ, et al: A genome-wide association meta-analysis identifies new childhood obesity loci. Nat Genet 2012;44:526–531.
13 Berndt SI, Gustafsson S, Magi R, et al: Genome-wide meta-analysis identifies 11 new loci for anthropometric traits and provides insights into genetic architecture. Nat Genet 2013;45:501–512.
14 Locke AE, Bratati K, Berndt SI, Justice AE, Pers TH, Day FR, Corey P, Sailaja V, Buchkovich ML, Jian Y: Genetic studies of body mass index yield new insights for obesity biology. Nature 2015;518:197–206.
15 Ioanna N, Kalliope P, Panagiota V, Lorraine S, Nigel WR, Eleftheria Z, Dedoussis GV: Replication of established common genetic variants for adult BMI and childhood obesity in Greek adolescents: the TEENAGE study. Ann Hum Genet 2013;77:268–274.
16 Hong KW, Oh B: Recapitulation of genome-wide association studies on body mass index in the Korean population. Int J Obes 2012;36:1127–1130.
17 Zhao J, Bradfield JP, Zhang H, Sleiman PM, Kim CE, Glessner JT, Deliard S, Thomas KA, Frackelton EC, Li M: Role of BMI-Associated Loci Identified in GWAS Meta-Analyses in the Context of Common Childhood Obesity in European Americans. Obesity (Silver Spring) 2011;19:2436–2439.
18 Hong J, Shi J, Qi L, Cui B, Gu W, Zhang Y, Li L, Xu M, Wang L, Zhai Y: Genetic susceptibility, birth weight and obesity risk in young Chinese. Int J Obesity 2013;37:673–677.
19 Wang HJ, Hinney A, Song JY, Scherag A, Meng XR, Grallert H, Illig T, Hebebrand J, Wang Y, Ma J: Association of common variants identified by recent genome-wide association studies with obesity in Chinese children: a case-control study. Bmc Med Genet 2016;17:7.
20 Meng XR, Song JY, Ma J, Liu FH, Shang XR, Guo XJ, Wang HJ: Association study of childhood obesity with eight genetic variants recently identified by genome-wide association studies. Pediatr Res 2014;76:310–315.
21 Wheeler E, Huang N, Bochukova EG, Keogh JM, Lindsay S, Garg S, Henning E, Blackburn H, Loos RJF, Wareham NJ, O'Rahilly S, Hurles ME, Barroso I, Farooqi IS: Genome-wide SNP and CNV analysis identifies common and low-frequency variants associated with severe early-onset obesity. Nat Genet 2013;45:513–517.
22 Wang HJ, Zhang H, Zhang SW, Pan YP, Ma J: Association of the common genetic variant upstream of INSIG2 gene with obesity related phenotypes in Chinese children and adolescents. Biomed Environ Sci 2008;21:528–536.
23 Wang D, Ma J, Zhang S, Hinney A, Hebebrand J, Wang Y, Wang HJ: Association of the MC4R V103I polymorphism with obesity: a Chinese case-control study and meta-analysis in 55,195 individuals. Obesity (Silver Spring) 2010;18:573–579.
24 Li BH, Wu SS, Li SS, Wang HJ, Ma J: Evaluation of school-based physical activity interventions for obesity in elementary students. Chin J School Health 2011;32:591–593.
25 Pei ZC, Wang HJ, Li BH, Wang JJ, Ma J: Effects of health education of dietary nutrition on primary students in Beijing. Chin J School Health 2011;32:779–780.
26 National Health and Family Planning Commission of PRC: WS/T 456–2014, Screening standard for malnutrition of school-age children and adolescents. China: NHFPC, 2014.
27 Ji CY: Report on childhood obesity in China (1) – body mass index reference for screening overweight and obesity in Chinese school-age children. Biomed Environ Sci 2005;18:390–400.
28 Li H, Zong XN, Ji CY, Mi J: Body mass index cut-offs for overweight and obesity in Chinese children and adolescents aged 2–18 years. Zhonghua Liu Xing Bing Xue Za Zhi 2010;31:616–620.
29 Chinese Working Group on Obesity for Children: Report on the Physical Fitness and Health Surveillance of Chinese School Students (2005). Beijing, Higher Education Press, 2007, pp 220–222.
30 World Health Organization: Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser 1995;854:1–452.
31 World Health Organization: Growth reference data for 5–19 years, *www.who.int/growthref/en/* (last accessed May 16, 2017).
32 National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents: The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114:555–576.
33 Weir BS, Cockerham CC: Estimating F-statistics for the analysis of population structure. Evolution 1984;38:1358–1370.
34 Duan S, Zhang W, Cox NJ, Dolan ME: FstSNP-HapMap3:a database of SNPs with high population differentiation for HapMap3. Bioinformation 2008;3:139–141.
35 Balloux F, Lugon-Moulin N: The estimation of population differentiation with microsatellite markers. Mol Ecol 2002;11:155–165.
36 Higgins JP, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analyses. Br Med J 2003;327:557–560.
37 Takkouche B, Cadarso-Suárez C, Spiegelman D: Evaluation of old and new tests of heterogeneity in epidemiologic meta-analysis. Am J Epidemiol 1999;150:206–215.

Obes Facts 2017;10:179–190
DOI: 10.1159/000471487

38 Schuurs-Hoeijmakers JH, Oh EC, Vissers LE, Swinkels ME, Gilissen C, Willemsen MA, Holvoet M, Steehouwer M, Veltman JA, de Vries BB, van Bokhoven H, de Brouwer AP, Katsanis N, Devriendt K, Brunner HG: Recurrent de novo mutations in PACS1 cause defective cranial-neural-crest migration and define a recognizable intellectual-disability syndrome. Am J Hum Genet 2012;91:1122–1127.
39 Wan L, Molloy SS, Thomas L, Liu G, Xiang Y, Rybak SL, Thomas G: PACS-1 defines a novel gene family of cytosolic sorting proteins required for trans-Golgi network localization. Cell 1998;94:205–216.
40 Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN: Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003;33:177–182.