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ORIGINAL ARTICLE

A polymorphism near osteoprotegerin gene confer risk of obesity in Uyghurs

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Abstract To investigate the association of single nucleotide polymorphism (SNP) rs4355801 near osteoprotegerin (OPG) gene and rs3736228 in low-density lipoprotein receptor-related protein 5 (LRP5) gene with metabolic phenotypes [body mass index (BMI), waist–hip ratio, glucose, total cholesterol (CHO), and triglyceride], we carried out a population-based association study in Uyghur population living in Xinjiang Uyghur Autonomous Region of China. We observed a significant higher level of BMI in AG/AA carriers than in GG carriers ($P = 0.022$) for rs4355801. Subjects with the AG/GG genotype significantly increased the risk of BMI related obesity than subjects with the AA genotype, with an odds ratio of 1.31 (95% CI 1.09–1.56, $P = 0.005$). The association remained significant after controlling for covariates of age and gender. In addition, we observed a significant higher level of CHO in CT/TT carriers than in CC carriers ($P = 0.021$) for rs3736228. Our observations provide the first evidence that rs4355801 near OPG gene may confer susceptibility to obesity. In addition, SNP rs3736228 in LPR5 gene may affects the level of CHO in Uyghur population.

Keywords Polymorphism · Association study · Osteoprotegerin · Obesity · Cholesterol

The Human Ethnics Committee of Fudan University approved the research.

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Introduction

In the past 2 years, there has been a dramatic increase in genomic discoveries involving complex, non-Mendelian diseases, with nearly 100 loci for as many as 40 common diseases robustly identified and replicated in genome-wide association (GWA) studies [1]. These GWA discoveries provide valuable clues to the allelic architecture of complex traits in general [2]. Recently, in a GWA study conducted in 8,557 Caucasian participants from twins UK cohort, the Rotterdam cohort, the Chingford cohort, and twins UK replication cohort, Richards et al. [3] identified a single nucleotide polymorphism (SNP) rs4355801, located in the 3′ untranslated region of the osteoprotegerin (OPG) gene, and a SNP rs3736228, located in the low-density lipoprotein receptor-related protein 5 (LRP5) gene, to be associated with bone mass density (BMD) and osteoporotic fractures. The risk A allele of rs4355801 and T allele of rs3736228, presented in 54 and 15% of the Caucasians, respectively, both increased the risk of osteoporotic fractures with a odds ratio (OR) of 1.3.

A number of studies have reported a correlation between bone mass phenotypes (e.g., BMD, osteoporotic fracture) and metabolic phenotypes (e.g., obesity, diabetes, and hyperlipidemia). In the Swedish population-based longitudinal prevention project which included 10,902 women, high body mass index (BMI) significantly increased the risk of proximal humerus and ankle fractures (RR 1.21–1.33) at an average of 15-year follow-up [4]. However, De Laet et al. [5] studied almost 60,000 subjects in a meta-analysis and found that the age-adjusted risk for any type of fracture increased significantly with lower BMI. The level of BMD is decreased in diabetes patients [6] and osteoporosis is seen in about 14–20% of patients with diabetes [7]. In a longitudinal study conducted in postmenopausal women aged 50–75 years, the greatest decreases in spine BMD was associated with the largest increases in serum cholesterol levels [8]. In the present study, we sought to investigate the relationship between the SNP rs4355801 and rs3736228, originally associated with BMD level and osteoporotic fracture in a GWA study conducted in Caucasians[3], and metabolic phenotypes [BMI, waist–hip ratio (WHR), glucose (Glu), triglyceride (TG), and total cholesterol (CHO)] in a minority population living in the northwest area of China—Uyghurs.

Methods

Subjects

This study included 770 Uyghur subjects (397 men and 373 women, mean age 55.46 ± 11.20 years) recruited in March to May 2005 and April 2006 in four villages of Tulupan District, Xinjiang Uyghur Autonomous Region of China. This study was designed to address the cardiovascular risk factors of the Uyghur farmers in the target population of 30 years or old. About 90–100 male and female subjects were randomly chosen from each village. Body weight and height were measured with subjects wearing only light indoor clothing and without shoes. BMI was calculated by dividing weight (kg) by height squared (m^2). Subjects recruited from the cross-sectional survey were further classified into obesity group (BMI $\geq$ 28 kg/m^2) and control group (18 $\leq$ BMI $<$ 24 kg/m^2) following the recommended obesity criteria for the Chinese population [9]. Waist and hip circumferences were measured to calculate WHR. Fasting blood specimen were drawn and analyzed for Glucose, TG, and CHO. Subjects were classified as diabetic if they had any previous diagnosis, history of antidiabetic medication use or fasting levels of plasma glucose $\geq$7 mmol/l. The distribution of clinical parameters was listed in Table 1. Written informed consents were obtained from all subjects in this study. The Human Ethnics Committee of Fudan University approved the research.

Table 1 Demographic characteristics of study population

Variable	All subjects
N/m/f	770/397/373 (51.6%/48.4%)
Age	55.46 ± 11.21 (30–90)
BMI	26.35 ± 4.52 (15.06–44.64)
WHR	0.90 ± 0.07 (0.57–1.14)
Glucose	5.78 ± 1.97 (3.19–22.88)
TG	1.56 ± 1.11 (0.25 + 12.1)
CHO	4.49 ± 1.09 (0.54–10.68)

Genotyping

Blood was taken into EDTA-containing receptacles and DNA samples were prepared from blood using a method described by Boom et al. [10]. Genotyping for rs3736228 and rs4355801 were accomplished using TaqMan assays (Applied Biosystems, Foster City, CA, USA). Fluorescence was detected using an ABI 7900HT and the alleles were scored by using Sequence Detection Software (Applied Biosystems, Foster City, CA, USA). The assay identifications of the Taqman probes of rs3736228 and rs4355801 were C_25752205_10 and C_11869235_10, respectively. Laboratory personnel were blinded to clinical status. In addition to quality control samples included in each batch by the laboratory, blinded quality control samples were included to monitor the reproducibility of the genotyping assays. The concordance of duplicate samples was >99%.

Statistical analysis

Deviation from Hardy–Weinberg expectation for the variants was tested by a chi-square statistic. Differences of mean clinical characteristics across genotype groups were tested by using analysis of variance (ANOVA) and analysis of covariance (ANCOVA) including gender and age as covariates. Allelic and genotypic frequencies were compared between the obesity subjects and controls by using a χ^2-test. Unconditional logistic regression model was used to estimate relative risk (RR) and their corresponding 95% confidence intervals (CI) adjusted for covariates gender and age. Owing to limited sample size, we dichotomized the genetic polymorphisms by grouping subjects into carriers and non-carriers of the risk allele in analysis. SPSS software version 13.0 for Windows was used in statistical evaluations of the above data.

Results

The genotyping success rates of rs4355801 and rs3736228 were 99.61 and 98.44%, respectively. Both rs4355801 and rs3736228 were consistent with Hardy–Weinberg expectations in Uyghur subjects. The frequencies of CC, CT, and TT genotypes and risk T allele of rs3736228 were 73.3, 25.2, 1.5, and 14.1%, respectively. Since the sample size of this study is relatively small, we compared the effects of risk allele carriers (AG/AA for rs4355801 and CT/TT for rs3736228) with the non-carriers on metabolic phenotypes in the follow analysis, as analyzed in the original GWA study [3].

For the SNP rs4355801, significant higher level of BMI was observed in AG/AA carriers (26.67 ± 4.38) than in GG carriers (25.90 ± 4.68) ($P = 0.022$). The association remained significant after controlling for age and genders ($P = 0.024$) (Table 2). Since diabetes status affected the level of BMI. We excluded the diabetic subjects ($n = 112$) when conducting the association study. We observed that significant higher level of BMI was also observed in AG/AA carriers (26.65 ± 4.39) than in GG carriers (25.80 ± 4.52) ($P = 0.017$) after excluding diabetes subjects. The association remained significant after controlling for age and genders ($P = 0.011$). For rs3736228, significant higher level of CHO was observed in CT/TT carriers (4.65 ± 1.18) than in CC carriers (4.44 ± 1.06) ($P = 0.021$). The association remained significant after controlling for age and genders ($P = 0.040$) (Table 2).

We further investigate the effect of the SNP rs4355801 on the predisposition of BMI related obesity after diabetic subjects were excluded. We observed that subjects with AG/GG genotype significantly increased the risk of obesity than subjects with AA genotype, with an odds ratio of 1.31 (95% CI 1.09–1.56, $P = 0.005$). The association remained significant after controlling for covariates age and gender (Table 3).

Discussion

In this study, we observed that A allele of the SNP rs4355801 increased the risk of obesity and the T allele of the SNP rs3736228 increased the level of CHO in Uyghur population. In particular, AG/AA genotypes of the rs4355801 had a 0.77 kg/m^2 mean BMI higher than GG genotype. Indeed, there are other literature examples where non-coding SNPs lead to a change in BMI of this magnitude. For example, Gaunt et al. [11] discovered that GG homozygote 1926C/G polymorphism in the 3'-UTR of the insulin-like growth factor 2 gene had a mean BMI 1.0 kg/m^2 lower than CC homozygotes ($P = 0.006$, $n = 1872$). To our knowledge, we are the first to report that rs4355801 in the 3'-UTR of the OPG gene is associated with obesity in a population.

LRP5 is widely expressed in several tissues including the hepatocytes, liver, pancreas, and adrenal glands. LRP5 plays an important part in the WNT signaling pathway [12], and is crucial in bone [13], cholesterol [14], and glucose metabolism [15]. The SNP rs3736228 (Ala1330-Val), located at the exon18 of the LPR5 gene, is a non-synonymous variation. It has been well documented that

Table 2 Clinical characteristics of subjects according to rs4355801 and rs3736228 genotypes in Uyghurs

Variable	rs4355801				rs3736228			
	GG ($N = 332$)	AG/AA ($N = 435$)	P	Adj-P	CC ($N = 556$)	CT/TT ($N = 202$)	P	Adj-P
BMI	25.90 ± 4.68	26.67 ± 4.38	0.022	0.024	26.29 ± 4.66	26.37 ± 4.13	0.825	0.958
WHR	0.897 ± 0.07	0.893 ± 0.07	0.732	0.684	0.897 ± 0.07	0.904 ± 0.07	0.304	0.260
Glucose	5.73 ± 1.66	5.82 ± 2.18	0.531	0.535	5.72 ± 1.74	5.93 ± 2.48	0.195	0.206
TG	1.51 ± 1.04	1.59 ± 1.16	0.301	0.339	1.54 ± 1.11	1.56 ± 1.09	0.831	0.955
CHO	4.52 ± 1.07	4.48 ± 1.11	0.629	0.522	4.44 ± 1.06	4.65 ± 1.18	0.021	0.040

N number of subjects, *Adj-P* adjusted for gender and age

Table 3 Odds ratios for BMI related obesity for rs4355801 in Uyghurs

Phenotype	Genotype frequency (%)		Crude RR	P	Adjusted RR	P
	GG	AG/AA	(95% CI)		(95% CI)	
Controls	99 (0.51)	95 (0.49)	1.33 (1.09–1.62)	0.005	1.33 (1.08–1.63)	0.007
Obesity	76 (0.37)	129 (0.63)				

Adjusted RR: Adjusted for age and sex

this SNP is associated with reduced BMD or increased risk of osteoporosis fracture [16–18]. Recently, SNPs rs3736228 was found associated with higher levels of total and low-density lipoprotein in CT carriers than in CC carriers in Finnish children [19]. Subjects with TT genotypes were also associated with higher risk of developing hypercholesterolemia in the general Japanese male population [20]. SNPs rs3736228 is functionally important since the T allele lead to decreased WNT signalling in vitro [21]. However, the mechanism that T allele of rs3736228 increase the level of cholesterol in Finnish children, the general Japanese male population, as well in our general Uyghur population need to be elucidated.

OPG is an osteoblast secreted decoy receptor that decreases bone resorption by inhibiting differentiation of osteoclast precursors and activation of mature osteoclasts, and by stimulating osteoclast apoptosis [22]. Serum OPG also affects the severity of coronary artery disease and peripheral artery disease [23, 24]. Polymorphisms in the OPG gene were associated with BMD [25], fracture [26], and coronary artery disease [27]. The SNP rs4355801 is located in the 3′ untranslated region of the OPG gene. Richards et al. [3] used *cis*-associated allelic expression study to ascertain whether A allele affected expression of OPG transcript in lymphoblast cell lines. In quantitative analysis of 27 samples with A alleles at rs4355801, the expression of OPG in lymphoblast cell lines was halved. The mechanism that AG/AA genotypes of rs4355801 linked to the increased risk of BMI related obesity in Uyghur population is still unknown. Since decreased level of BMI was associated with decreased level of BMD in most observations, and since A allele of rs4355801 is associated with decreased level of serum OPG, one could speculate that AG/AA genotypes of rs4355801 be associated with a decreased risk of obesity, instead of an increased risk of obesity in Uyghurs. However, the pathological process of obesity is so complex that it involves the interplay of many different molecules. It seems too early to make an isolate correlation between OPG levels and obesity since OPG is produced by a variety of tissues and regulated by various cytokines, peptides, and hormones [28, 29]. Holecki et al. [30] observed that serum concentration of OPG was significantly lower in obese patients in comparison with normal-weight controls in perimenopausal women. They also observed that weight reduction therapy resulted in further decrease in OPG serum concentrations. The association between rs4355801 and obesity in Uyghur population need to be replicated in other population.

The major strength of this study lies in its population-based design. The control subjects for obesity were selected randomly from the same base population from which the case subjects were derived. It overcomes the potential selection bias associated with hospital-based designs. Remarkably, the Uyghur population we studied is a classic admixed population with a genetic background of Caucasian (42.6%) and Asian (57.4%) [31, 32]. The increased extent of LD between markers on the same chromosome, created by population admixture, may facilitate genome mapping of complex disease genes for Uyghur population [32]. The Uyghur population lived in Tulupan area is ethnically homogeneous population with respect to a variety of factors such as social structure, profession, living environment, and lifestyle between hypertensive and control subjects, thus limiting the effect of population stratification on our results. However, the study sample size is relatively small, which decreased the power of genetic associations; thus chance findings cannot be fully excluded. Since the results of genetic association studies should be considered robust only if they are replicated in independent groups and independent populations, the preliminary finding that OPG rs4355801 is associated with obesity in Uyghurs needs to be replicated in other studies and other ethnic groups. The biological mechanism underlying this association also needs exploring.

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