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Common Variants in *CDKAL1*, *CDKN2A/B*, *IGF2BP2*, *SLC30A8*, and *HHEX/IDE* Genes Are Associated With Type 2 Diabetes and Impaired Fasting Glucose in a Chinese Han Population

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OBJECTIVE—Genome-wide association studies have identified common variants in *CDKAL1*, *CDKN2A/B*, *IGF2BP2*, *SLC30A8*, *HHEX/IDE*, *EXT2*, and *LOC387761* loci that significantly increase the risk of type 2 diabetes. We aimed to replicate these observations in a population-based cohort of Chinese Hans and examine the associations of these variants with type 2 diabetes and diabetes-related phenotypes.

RESEARCH DESIGN AND METHODS—We genotyped 17 single nucleotide polymorhisms (SNPs) in 3,210 unrelated Chinese Hans, including 424 participants with type 2 diabetes, 878 with impaired fasting glucose (IFG), and 1,908 with normal fasting glucose.

RESULTS—We confirmed the associations between type 2 diabetes and variants near *CDKAL1* (odds ratio 1.49 [95% CI 1.27–1.75]; $P = 8.91 \times 10^{-7}$) and *CDKN2A/B* (1.31 [1.12–1.54]; $P = 1.0 \times 10^{-3}$). We observed significant association of SNPs in *IGF2BP2* (1.17 [1.03–1.32]; $P = 0.014$) and *SLC30A8* (1.12 [1.01–1.25]; $P = 0.033$) with combined IFG/type 2 diabetes. The SNPs in *CDKAL1*, *IGF2BP2*, and *SLC30A8* were also associated with impaired β-cell function estimated by homeostasis model assessment of β-cell function. When combined, each additional risk allele from *CDKAL1*-rs9465871, *CDKN2A/B*-rs10811661, *IGF2BP2*-rs4402960, and *SLC30A8*-rs13266634 increased the risk for type 2 diabetes by 1.24-fold ($P = 2.85 \times 10^{-7}$) or for combined IFG/type 2 diabetes by 1.21-fold ($P = 6.31 \times 10^{-11}$). None of the SNPs in *EXT2* or *LOC387761* exhibited significant association with type 2 diabetes or IFG. Significant association was observed between the *HHEX/IDE* SNPs and type 2 diabetes in individuals from Shanghai only ($P < 0.013$) but not in those from Beijing ($P > 0.33$).

CONCLUSIONS—Our results indicate that in Chinese Hans, common variants in *CDKAL1*, *CDKN2A/B*, *IGF2BP2*, and *SLC30A8* loci independently or additively contribute to type 2 diabetes risk, likely mediated through β-cell dysfunction. ***Diabetes*** **57:2834–2842, 2008**

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Received 14 January 2008 and accepted 3 July 2008.

Published ahead of print at http://diabetes.diabetesjournals.org on 15 July 2008. DOI: 10.2337/db08-0047.

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The rapid increase in prevalence of type 2 diabetes has been a major public health challenge worldwide, including China. The total number of people with diabetes in China is estimated to increase from 20.8 million in 2000 to 42.3 million in 2030 (1). Besides the important contribution of environmental factors, including changes in dietary patterns and lifestyle, genetic determinants also play a major role in type 2 diabetes susceptibility. Over the past decade, serious efforts have been put into the search for type 2 diabetes susceptibility genes, but progress has been slower than anticipated (2,3). Although common variants in a few genes including *PPARG*, *KCNJ11*, and *TCF7L2* have been convincingly replicated in individuals with European ancestry, relatively few studies have been conducted in Chinese, and, so far, no variants have been unambiguously confirmed as diabetes susceptibility loci in Chinese. However, recent advances in genome-wide association studies (GWASs) have revived the initial optimism and accelerated the discovery of diabetes susceptibility genes (4–6).

The first GWAS, conducted in a French case-control cohort, confirmed *TCF7L2* as a major type 2 diabetes susceptibility gene and identified four novel loci consistently associated with type 2 diabetes (7). These loci are located in chromosomal regions that harbor several genes involved in β-cell function or development, including a variant in the *SLC30A8* (zinc transporter solute carrier family 30 member 8) gene, variants located in a linkage disequilibrium (LD) block that contains the *IDE* (insulin-degrading enzyme), *KIF11* (kinesin family member 11), and the *HHEX* (hematopoietically expressed homeobox) genes, as well as variants in another LD block that contains genes encoding *EXT2* (exostosin 2). A fourth locus mapped to a hypothetical gene *LOC387761* on chromosome 11. Four subsequent GWASs (8–12), performed in European case-control studies, confirmed the *SLC30A8* and *HHEX/IDE* genes as type 2 diabetes susceptibility loci. Furthermore, additional variants in several new gene regions were also identified, including single nucleotide polymorhisms (SNPs) in the *CDKAL1* gene, which encodes the *CDK5* regulatory subunit associated protein 1-like 1; in the *CDKN2A/B* genes, which encode the cyclin-dependent kinase inhibitor $p15^{INK4a}$ and $p16^{INK4b}$; in the *IGF2BP2* gene, which encodes the *IGF-2* mRNA binding protein 2; and a variant in a region of chromosome 11, not known to contain any genes. Most of these newly identified loci are suggested to play a role in the regulation

TABLE 1
Characteristics of the study population

	All samples	Beijing	Shanghai	*P*
n (% male)	3,210 (44.3)	1,574 (45.2)	1,636 (43.5)	
Age (years)	58.6 ± 6.0	58.3 ± 5.9	58.9 ± 6.0	0.0095
BMI (kg/m^2)	24.2 (22.0–26.6)	25.1 (22.8–27.4)	23.5 (21.3–25.9)	<0.0001
Fasting glucose (mmol/l)	5.84 ± 1.74	6.16 ± 1.96	5.53 ± 1.42	<0.0001
A1C (%)	5.99 ± 1.10	6.08 ± 1.22	5.90 ± 0.96	<0.0001
Fasting insulin (pmol/l)	82.2 (59.4–112.2)	81.0 (57.6–110.7)	84.0 (61.8–114.0)	0.0777
HOMA-B (%)	110.3 ± 47.0	100.1 ± 44.9	120.0 ± 46.9	<0.0001
HOMA-S (%)	63.7 (47.1–86.9)	64.0 (47.3–89.5)	63.5 (46.9–85.1)	0.0454
IFG (%)	878 (27.4)	579 (36.8)	299 (18.3)	<0.0001
Type 2 diabetes (%)	424 (13.2)	272 (17.3)	152 (9.3)	<0.0001

Data are means ± SD, median (interquartile range), or *n* (%), unless otherwise indicated. *P* represents significance of the differences between individuals from Beijing and from Shanghai.

of insulin production and β-cell function (5,7,9,12–15). It is unclear whether these variants have the same effect in Chinese populations, which have a different genetic background and lower diabetes prevalence compared with European populations (16–18).

Although case-control studies provide a useful design for the discovery of susceptibility loci, they are limited in providing insight into the mechanisms through which genetic variants exert their effect on the risk of type 2 diabetes. Population-based cohort studies with detailed measures of diabetes-related traits, however, might unravel the physiopathology that underlies the association between the newly discovered genetic variants and diabetes. The purpose of this study is to examine whether these novel variants are individually or collectively associated with type 2 diabetes and related traits in a population-based Chinese Han cohort including 3,210 unrelated individuals from Beijing and Shanghai.

RESEARCH DESIGN AND METHODS

The study sample consisted of 3,210 individuals (1,423 men and 1,787 women) from the Study on Nutrition and Health of Aging Population in China. The study population, design, and protocols of this population-based cohort study have been previously described (19). Briefly, all participants were unrelated Chinese Hans, aged 50–70 years, with at least 20 years residence in Beijing or Shanghai. Among them, 424 participants had type 2 diabetes (267 had previously diagnosed type 2 diabetes and 157 had screen-detected and treatment-naive type 2 diabetes), 878 participants had impaired fasting glucose (IFG) (all 878 were screen detected and treatment naive), and 1,908 participants had normal fasting glucose (NFG). Type 2 diabetes was defined by either 1999 World Health Organization criteria (20) or previously diagnosed type 2 diabetes. NFG and IFG were defined as fasting glucose <5.6 mmol/l (100 mg/dl) and 5.6 mmol/l (100 mg/dl) less than or equal to fasting glucose <7.0mmol/l (126 mg/dl), respectively. The study was conducted simultaneously in both Beijing and Shanghai from March to June 2005. The participants were recruited from two urban districts (400 participants for each district) and one rural district (800 participants), representing people with high to low socioeconomic status, using a multistage sampling method in each city. All participants were selected randomly from the eligible candidates listed in the residential registration record. One person from each household was allowed to participate, and at least 40% of the total participants were men in each district. Individuals with the following conditions were excluded from the study: *1*) severe psychological disorders, physical disabilities, cancer, cardiovascular disease, Alzheimer's disease, or dementia, within 6 months; or *2*) currently diagnosed with tuberculosis, AIDS, and other communicable diseases. All participants attended a physical examination, during which standard anthropometric measurements and overnight fasting blood samples were collected. Glucose was measured enzymatically on an automatic analyzer (Hitachi 7080; Hitachi, Tokyo, Japan) with reagents purchased from Wako Pure Chemical Industries (Osaka, Japan). Fasting insulin was determined by radioimmunoassay (Linco Research, St. Charles, MO). A1C concentrations were measured by turbidometric immunoassay in red blood cells on the Hitachi 7080 Analyzer using reagents from Roche Diagnostics (Indianapolis, IN). Homeostasis model assessment (HOMA) of insulin sensitivity (HOMA-S) and β-cell function (HOMA-B) were estimated using Levy's computer model (21). Written informed consent was obtained from all participants, and study protocols were approved by the institutional review board of the Institute for Nutritional Sciences. The phenotypic characteristics of the population are shown in Table 1.

Genotyping. Genomic DNA was extracted from peripheral blood leukocytes by the salting-out procedure (available at http://humgen.wustl.edu/hdk_lab_manual/dna/dna2.html). SNP genotyping was performed with the GenomeLab SNPstream Genotyping System (Beckman Coulter), according to the manufacturer's protocol. Seventeen SNPs previously reported to be associated with type 2 diabetes by at least one of the GWASs (7–12) were successfully genotyped in our population. These include SNPs near *CDKAL1* (rs10946398, rs7754840, rs7756992, and rs9465871), *HHEX/IDE* (rs1111875, rs5015480, and rs7923837), *EXT2* (rs1113132, rs11037909, and rs3740878), *CDKN2A/B* (rs10811661 and rs564398), *IGF2BP2* (rs4402960 and rs1470579), *SLC30A8* (rs13266634) (R325W), *LOC387761* (rs7480010), and an intergenic SNP (rs9300039) in chromosome 11. The genotyping success rate was >97.1%, and the concordance rate was >99% based on 12% duplicate samples ($n = 384$). Samples with ambiguous base calling were genotyped again. Genotype frequencies of all 17 SNPs were consistent with Hardy-Weinberg equilibrium ($P > 0.01$), and most of the minor allele frequencies observed in this study were comparable with those in the HapMap CHB (Chinese Han in Beijing) sample (online appendix Table 1 [available at http://dx.doi.org/10.2337/db08-0047]). Genotypic distributions were similar in Beijing and Shanghai populations ($P > 0.05$), except for the three *HHEX* SNPs ($P = 0.041$, 0.003, and 0.005 for rs1111875, rs5015480, and rs7923837, respectively).

Statistical analyses. Hardy-Weinberg equilibrium was tested using a likelihood ratio test. LD between SNPs was estimated using Haploview version 3.2 (available at http://www.broad.mit.edu/mpg/haploview). The association between each SNP and the risk of type 2 diabetes and IFG was examined using logistic regression. Generalized linear regression was applied to study the associations between each SNP and type 2 diabetes–related quantitative traits. Participants with known diabetes or receiving glucose-lowering treatment ($n = 267$) were excluded from the type 2 diabetes–related quantitative trait analyses. All association analyses assumed an additive effect of the risk allele and were adjusted for sex, age, BMI (where appropriate), and geographical region (Shanghai versus Beijing). BMI, insulin, and HOMA-S were log transformed before analyses, and the data were presented as geometric means. Likelihood ratio tests were used to examine genotype distribution in Beijing and Shanghai. Because of a significant difference in genotype distribution of the three *HHEX/IDE* SNPs ($P < 0.05$) and in diabetes prevalence between the Shanghai and Beijing participants ($P < 0.0001$), analyses for these SNPs were performed for Shanghai and Beijing separately.

Gene-gene interactions were assessed by including the respective interaction terms of pairwise SNPs in logistic regressions using the maximum likelihood estimation. The combined effect of multiple SNPs on the risk of type 2 diabetes and/or IFG was determined by logistic regression after categorizing the participants into groups according to the number of the risk alleles they carried. Participants with one or no risk alleles served as the reference group. Bonferroni correction was used to adjust for multiple testing in the quantitative trait analyses. Association analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC). Meta-analyses were conducted with Stata (version 9.2; Stata, College Station, TX). Cochran's *Q* test was performed to assess heterogeneity among different groups. Power calculations were

TABLE 2
Associations with type 2 diabetes or IFG and type 2 diabetes combined

SNP identification	Gene	Major/minor allele*	Type 2 diabetes vs. normal: Minor allele frequency Case	Control	Odds ratio (95% CI)	$P_{(add)}$	Type 2 diabetes and IFG vs. normal: Minor allele frequency Case	Control	Odds ratio (95% CI)	$P_{(add)}$
All samples										
rs10946398	*CDKAL1*	A/**C**	0.500	0.409	1.47 (1.25–1.73)	2.32×10^{-6}	0.457	0.409	1.20 (1.07–1.33)	0.0012
rs7754840	*CDKAL1*	G/**C**	0.501	0.407	1.49 (1.27–1.75)	8.91×10^{-7}	0.459	0.407	1.22 (1.10–1.36)	0.0003
rs7756992	*CDKAL1*	**G**/A	0.426	0.497	1.38 (1.17–1.62)	9.35×10^{-5}	0.454	0.497	1.21 (1.09–1.35)	0.0004
rs9465871	*CDKAL1*	**C**/T	0.415	0.493	1.41 (1.21–1.66)	1.80×10^{-5}	0.449	0.493	1.21 (1.09–1.35)	0.0003
rs10811661	*CDKN2A/B*	**T**/C	0.418	0.483	1.31 (1.12–1.54)	0.0010	0.432	0.483	1.26 (1.13–1.41)	2.76×10^{-5}
rs564398	*CDKN2A/B*	**T**/C	0.131	0.128	1.07 (0.84–1.26)	0.59	0.135	0.128	0.99 (0.85–1.16)	0.92
rs4402960	*IGF2BP2*	G/**T**	0.264	0.241	1.14 (0.95–1.35)	0.16	0.263	0.241	1.17 (1.03–1.32)	0.014
rs1470579	*IGF2BP2*	A/**C**	0.272	0.246	1.15 (0.97–1.38)	0.11	0.268	0.246	1.17 (1.03–1.32)	0.013
rs13266634	*SLC30A8*	**C**/T	0.417	0.432	1.09 (0.93–1.27)	0.28	0.411	0.432	1.12 (1.01–1.25)	0.033
rs1113132	*EXT2*	**C**/G	0.390	0.418	1.12 (0.96–1.32)	0.15	0.410	0.418	1.04 (0.93–1.15)	0.53
rs11037909	*EXT2*	**T**/C	0.381	0.418	1.16 (0.99–1.36)	0.07	0.406	0.418	1.04 (0.94–1.16)	0.43
rs3740878	*EXT2*	**A**/G	0.405	0.431	1.11 (0.95–1.31)	0.19	0.418	0.431	1.06 (0.95–1.18)	0.29
rs7480010	*LOC387761*	A/**G**	0.223	0.225	0.98 (0.80–1.18)	0.79	0.230	0.225	1.00 (0.88–1.13)	0.97
rs9300039	Unknown	**C**/A	0.279	0.274	0.96 (0.80–1.14)	0.62	0.265	0.274	1.06 (0.94–1.19)	0.34
Beijing										
rs1111875	*HHEX*	A/**G**	0.306	0.309	1.00 (0.81–1.25)	0.94	0.279	0.309	0.89 (0.76–1.04)	0.13
rs5015480	*HHEX*	**T**/**C**	0.202	0.185	1.13 (0.88–1.46)	0.33	0.173	0.185	0.94 (0.78–1.13)	0.52
rs7923837	*HHEX*	A/**G**	0.244	0.231	1.09 (0.86–1.39)	0.48	0.229	0.231	1.01 (0.84–1.20)	0.95
Shanghai										
rs1111875	*HHEX*	A/**G**	0.376	0.276	1.64 (1.25–2.15)	0.0004	0.294	0.276	1.10 (0.92–1.32)	0.30
rs5015480	*HHEX*	T/**C**	0.218	0.138	1.79 (1.30–2.47)	0.0003	0.183	0.138	1.43 (1.15–1.78)	0.0013
rs7923837	*HHEX*	A/**G**	0.252	0.186	1.45 (1.08–1.94)	0.0131	0.231	0.186	1.30 (1.07–1.58)	0.0089

Odds ratios represent the effects of risk alleles. The *P* values were adjusted for age, sex, BMI, and region (where appropriate). *Alleles in bold are the risk alleles for type 2 diabetes identified by previous studies, while alleles underlined are the risk alleles for type 2 diabetes or IFG observed in this study. All analyses were based on an additive model, in which individuals homozygous for the nonrisk alleles were coded as 0, heterozygous individuals were coded as 1, and individuals homozygous for the risk alleles were coded as 2.

performed using Quanto software (available at http://hydra.usc.edu/gxe/), and the power shown in online appendix Table 1 was calculated for association between each SNP and type 2 diabetes using the odds ratios (7–12) reported in the original studies and sample size and minor allele frequencies in our own study.

RESULTS

We first examined the association with the risk of type 2 diabetes and IFG (Table 2). The four *CDKAL1* SNPs spanned two LD blocks (r^2 = 1.0 for rs7754840 and rs10946398 and 0.96 for rs7756992 and rs9465871) and were each significantly associated with type 2 diabetes (odds ratios ranged between 1.38 and 1.49; $P < 1.9 \times 10^{-5}$) and with combined IFG/type 2 diabetes (between 1.20 and 1.22; $P < 0.0013$). The *CDKN2A/B* rs10811661 variant was also associated with type 2 diabetes (odds ratio 1.31 [95% CI 1.12–1.54]; $P = 0.001$) and combined IFG/type 2 diabetes (1.26 [1.13–1.41]; $P = 2.76 \times 10^{-5}$). The second *CDKN2A/B* SNP (rs564398), which was not in LD with rs10811661 ($r^2 = 0$), was not associated with type 2 diabetes or combined type 2 diabetes/IFG. The two SNPs in *IGF2BP2* ($r^2 = 0.83$) and the *SLC30A8* SNP (rs13266634) showed modest association with combined IFG/type 2 diabetes (odds ratios between 1.12 and 1.17; $P = 0.013$–0.033) but not with type 2 diabetes alone.

The three *EXT2* variants were in complete LD ($r^2 = 1.0$) and occurred less frequently in our population (58%) than in European populations (70%). These variants, as well as those in chromosome 11 (rs7480010 and rs9300039, $r^2 = 0.037$), were not associated with type 2 diabetes or IFG. Analyses for the three SNPs in the *HHEX/IDE* LD block were performed separately in Shanghai and Beijing populations, as the difference in genotype distribution and prevalence of type 2 diabetes and IFG could lead to spurious associations due to population stratification (Table 2). All three *HHEX/IDE* SNPs were significantly associated with type 2 diabetes in Shanghai participants, with rs5015480 and rs7923837 also associated with combined IFG/type 2 diabetes. Meta-analyses suggested that the associations exhibited significant heterogeneity for SNPs rs1111875 ($P = 0.006$) and rs5015480 ($P = 0.028$) between Beijing and Shanghai populations.

We next examined the association between genetic variants and type 2 diabetes–related quantitative traits (glucose, A1C, insulin, HOMA-B, HOMA-S, and BMI) to investigate whether these variants conferred risk of type 2 diabetes through their effects on any of these intermediate traits (Table 3). Consistent with the case-control analyses, the SNPs that showed significant evidence for association with diabetes-related phenotypes were those that were also associated with type 2 diabetes or IFG, except for *CDKN2A/B* rs10811661 and *LOC387761* rs7480010. All four *CDKAL1* SNPs were significantly associated with A1C (*P* values 0.036–0.0096) and HOMA-B (*P* values 0.024–0.0009). The SNPs (rs7756992 and rs9465871) in the second LD block of this locus also showed significant association with fasting glucose levels ($P < 0.04$). Interestingly, the allele of *SLC30A8* SNP rs13266634 that increases the risk of combined IFG/type 2 diabetes was significantly associated with lower BMI ($P = 0.0087$) and marginally associated with decreased HOMA-B ($P = 0.05$). Only the associations of *CDKAL1*-rs10946398, rs7754840, and *IGF2BP2*-rs4402960 with HOMA-B remained signifi-

cant after Bonferroni correction for multiple testing ($P = 0.0014$, 0.05/36 tests).

To examine whether the associations for the *CDKAL1* variants were independent, we performed additional multiple regression analyses that included all four *CDKAL1* SNPs in one model. Results showed that none of the four SNPs remained significant ($P \geq 0.17$). Next, we tested whether the two *CDKAL1* "pairs" (rs7754840 and rs7756992 were chosen to represent each of the pairs) were independent from each other for the associations with type 2 diabetes or related quantitative traits in multiple regression models with both rs7754840 and rs7756992 genotypes in the model, with age, sex, region, and BMI (where appropriate) as covariates. The results revealed that the association seems to be driven by rs7754840, for the associations with type 2 diabetes, BMI, and HOMA-B, or by rs7756992, for the association with A1C, but interestingly, rs7754840 and rs7756992 seem to have independent effects on the associations with HOMA-S or insulin (online appendix Table 2).

We also performed a meta-analysis with the data from the previously published studies (10–12), including those from Japanese, Korean, and Hong Kong Chinese populations (22–25), to assess the heterogeneity between Caucasians and Asians for the *CDKAL1* and *CDKN2A/B* loci (rs7754840 and rs10811661 were chosen to represent each of them, respectively). The results showed that for the *CDKN2A/B* loci (rs10811661), the heterogeneity between Caucasians and Asians did not reach significance ($P = 0.059$), while a significant heterogeneity was observed between Caucasians and Asians ($P = 8.872 \times 10^{-6}$) for the *CDKAL1* loci (rs7754840) (online appendix Fig. 1), and this is consistent with the recent finding reported by Ng et al. (25).

Although we did not observe the association among the *LOC387761* SNP rs7480010 and type 2 diabetes or IFG, we found that the allele that increased the diabetes risk in European populations was modestly associated ($P < 0.03$) with increased insulin sensitivity (HOMA-S) and lower fasting insulin levels. Furthermore, despite a strong association between the *CDKN2A/B* SNP rs10811661 and type 2 diabetes, no association was observed with any of the diabetes-related quantitative traits. The intergenic SNP rs9300039 and the three *EXT2* SNPs (rs3740878, rs11037909, and rs1113132) were not associated with any of the diabetes-related quantitative traits.

We found no evidence of multiplicative gene-gene interactions among the main SNPs (rs9465871, rs10811661, rs4402960, and rs13266634) in each of the *CDKAL1*, *CDKN2A/B*, *IGF2BP2*, and *SLC30A8* genes. A significantly higher proportion of participants with type 2 diabetes carry increasing numbers of risk alleles, compared with participants with NFG (Fig. 1*A*). In combined analysis, each additional risk allele increased the risk of type 2 diabetes by 1.24-fold ($P = 2.85 \times 10^{-7}$) (Fig. 1*B*) and combined IFG/type 2 diabetes by 1.21-fold ($P = 6.31 \times 10^{-11}$) (Fig. 1*C*). Participants harboring seven or all eight risk alleles had a 4.44-fold increased risk for type 2 diabetes ($P = 5 \times 10^{-4}$) compared with those with one or no risk alleles (Fig. 1*B*). Consistently, participants with increasing numbers of risk alleles tended to have increased fasting levels of plasma glucose ($P = 0.013$) (Fig. 1*D*) and A1C ($P = 0.07$) (Fig. 1*E*), as well as decreased HOMA-B values ($P = 3.34 \times 10^{-7}$) (Fig. 1*F*). Of note, participants with increasing numbers of risk alleles tended to have significantly lower BMI ($P = 5.3 \times 10^{-3}$) (Fig. 1*F*), which is consistent with previous results found for the *CDKAL1* and *SLC30A8* polymorphisms (Table 3).

DISCUSSION

In this study of Chinese Hans, we replicated associations with several diabetes susceptibility variants recently identified through GWASs in white Europeans (7–12). Variants in *CDKAL1*, *CDKN2A/B*, *IGF2BP2*, *SLC30A8*, and *HHEX* loci were significantly associated with the risk of type 2 diabetes or combined IFG/type 2 diabetes. Furthermore, variants in *CDKAL1* and *IGF2BP2* were strongly associated with β-cell function estimated by HOMA-B.

The risk alleles of the *CDKAL1* variants increased diabetes risk by ~1.4-fold. These associations were stronger than those observed in individuals of European Ancestry (8–10,12) (online appendix Table 1), and *CDKAL1* risk allele frequencies are also substantially higher in Chinese (43–55%) than Europeans (15–31%). Moreover, significant heterogeneity between Caucasians and Asians was found for the *CDKAL1* loci (rs7754840) in the meta-analysis that combined the data from the previous studies in white Europeans, Japanese, Korean, Hong Kong Chinese, and our study ($P = 8.872 \times 10^{-6}$) (online appendix Fig. 1), while no significant heterogeneity was observed among the Asians ($P = 0.369$). These observations suggest that these *CDKAL1* variants might play an even more important role in diabetes susceptibility in Chinese. The risk allele of the first pair of *CDKAL1* variants was strongly associated with reduced β-cell function (HOMA-B) and increased A1C levels, while the second pair of *CDKAL1* variants showed an association with impaired β-cell function (HOMA-B) and higher glucose levels, as well as with increased A1C. The results from additional multiple regression analyses suggest that the four SNPs most likely represent the effects of a single *CDKAL1* locus. However, none of these four SNPs stands out as being the variant driving the association. Therefore, we assume that none of them is likely to be the causal variant, but presumably they are in moderate to high LD with the causal SNP and are therefore less consistently associated with the traits of interest. This region would benefit from a detailed fine mapping to identify possible causal variants in future studies. These results support previous findings (9,13,26) that the four *CDKAL1* SNPs confer the risk of type 2 diabetes through reduced insulin secretion, although the causal SNP is yet to be identified.

We also observed significant association between *CDKN2A/B* rs10811661 and type 2 diabetes and IFG with a slightly higher odds ratio (~1.3) than that observed in Europeans (~1.20) (10–12). The risk allele is twice as prevalent in Chinese Hans (46%) as in Europeans (21%). However, we did not observed significant heterogeneity between Caucasians and Asians in the meta-analysis with data from the previously published studies ($P = 0.059$). Interestingly, none of the diabetes-related traits showed an association with *CDKN2A/B* rs10811661. The second *CDKN2A/B* variant, rs564398, which is less frequent in Chinese Hans (13%) than in Europeans (38%), was not associated with type 2 diabetes or any related traits.

The association between variants in *IGF2BP2* and type 2 diabetes was not significant, although the odds ratios were similar to those observed in European populations (~1.15), suggesting that our study may not have been sufficiently powered. Indeed, assuming an additive model and a minor allele frequency of 25%, we had <50% power

TABLE 3
Associations with type 2 diabetes–related quantitative traits

SNP identification (major/minor allele)	n	Glucose (mmol/l)* Means ± SE	P‡	A1C (%)* Means ± SE	P‡	Insulin (pmol/l)† Means ± SE	P‡	HOMA-B (%)* Means ± SE	P‡	HOMA-S (%)† Means ± SE	P‡	BMI (kg/m^2)† Means ± SE	P§
CDKAL1													
rs10946398 (A/**C**)													
Genotype													
AA	972	5.53 ± 0.04		5.78 ± 0.03		80.4 ± 1.3		115.9 ± 1.4		65.4 ± 1.0		24.1 ± 0.1	
AC	1,429	5.62 ± 0.03	0.10	5.85 ± 0.02	0.036	78.7 ± 1.0	0.11	112.8 ± 1.1	0.0009¶	66.6 ± 0.8	0.10	24.2 ± 0.1	0.16
CC	516	5.62 ± 0.05		5.85 ± 0.04		77.1 ± 1.7		99.4 ± 1.8		68.2 ± 1.4		23.8 ± 0.1	
rs7754840 (G/**C**)													
Genotype													
GG	975	5.52 ± 0.04		5.77 ± 0.03		80.4 ± 1.3		115.8 ± 1.4		65.5 ± 1.0		24.2 ± 0.1	
GC	1,443	5.61 ± 0.03	0.09	5.85 ± 0.02	0.034	79.1 ± 1.0	0.08	113.3 ± 1.1	0.0011¶	66.3 ± 0.8	0.07	24.2 ± 0.1	0.10
CC	514	5.62 ± 0.05		5.85 ± 0.04		76.6 ± 1.6		107.9 ± 1.9		68.7 ± 1.4		23.8 ± 0.2	
rs7756992 (**G**/A)													
Genotype													
GG	774	5.68 ± 0.04		5.88 ± 0.03		79.2 ± 1.4		109.6 ± 1.5		66.7 ± 1.1		24.0 ± 0.1	
GA	1,455	5.56 ± 0.03	0.035	5.82 ± 0.02	0.011	79.0 ± 1.0	0.62	114.1 ± 1.1	0.024	66.6 ± 0.8	0.84	24.2 ± 0.1	0.69
AA	681	5.55 ± 0.05		5.77 ± 0.03		78.2 ± 1.5		114.6 ± 1.6		66.8 ± 1.2		24.1 ± 0.1	
rs9465871 (**C**/T)													
Genotype													
CC	805	5.67 ± 0.04		5.88 ± 0.03		78.8 ± 1.4		109.9 ± 1.5		66.5 ± 1.1		24.0 ± 0.1	
CT	1,422	5.57 ± 0.03	0.035	5.82 ± 0.02	0.0096	78.9 ± 1.0	0.72	113.5 ± 1.1	0.0065	66.8 ± 0.8	0.65	24.2 ± 0.1	0.59
TT	688	5.54 ± 0.04		5.77 ± 0.03		79.5 ± 1.5		115.9 ± 1.6		65.7 ± 1.2		24.1 ± 0.1	
CDKN2A/B													
rs10811661 (**T**/C)													
Genotype													
TT	813	5.60 ± 0.04		5.81 ± 0.03		78.3 ± 1.3		110.7 ± 1.5		67.2 ± 1.1		24.0 ± 0.1	
TC	1,489	5.59 ± 0.03	0.48	5.84 ± 0.02	0.73	80.3 ± 1.0	0.70	113.9 ± 1.1	0.09	65.5 ± 0.8	0.85	24.0 ± 0.1	0.39
CC	620	5.55 ± 0.05		5.82 ± 0.03		77.2 ± 1.5		114.4 ± 1.7		67.8 ± 1.3		24.2 ± 0.1	
rs564398 (**T**/C)													
Genotype													
TT	2,173	5.58 ± 0.03		5.82 ± 0.02		78.6 ± 0.8		112.5 ± 0.9		66.9 ± 0.7		24.0 ± 0.1	
TC	694	5.62 ± 0.05	0.55	5.83 ± 0.03	0.73	80.9 ± 1.5	0.22	114.5 ± 1.6	0.31	65.8 ± 1.1	0.19	24.2 ± 0.1	0.51
CC	40	5.58 ± 0.19		5.92 ± 0.13		78.4 ± 6.1		113.4 ± 6.7		67.3 ± 5.0		23.5 ± 0.5	
IGF2BP2													
rs4402960 (G/**T**)													
Genotype													
GG	1,635	5.54 ± 0.03		5.81 ± 0.02		80.3 ± 1.0		115.4 ± 1.1		65.5 ± 0.8		24.1 ± 0.1	
GT	1,108	5.64 ± 0.04	0.037	5.85 ± 0.02	0.33	70.0 ± 1.0	0.06	110.2 ± 1.3	0.0005¶	67.8 ± 1.0	0.07	24.1 ± 0.1	0.31
TT	173	5.67 ± 0.09		5.83 ± 0.06		77.5 ± 2.9		108.0 ± 3.2		67.7 ± 2.4		23.8 ± 0.3	
rs1470579 (A/**C**)													
Genotype													
AA	1,597	5.54 ± 0.03		5.80 ± 0.02		80.0 ± 1.0		115.3 ± 1.1		65.8 ± 0.8		24.2 ± 0.1	
AC	1,143	5.64 ± 0.04	0.029	5.85 ± 0.02	0.11	78.2 ± 1.1	0.17	111.0 ± 1.3	0.0026	67.1 ± 0.9	0.20	24.0 ± 0.1	0.23
CC	167	5.66 ± 0.10		5.86 ± 0.06		77.1 ± 2.9		108.2 ± 3.3		68.1 ± 2.5		23.9 ± 0.3	

TABLE 3
Continued

SNP identification (major/minor allele)	n	Glucose (mmol/l)* Means ± SE	P‡	A1C (%)* Means ± SE	P‡	Insulin (pmol/l)† Means ± SE	P‡	HOMA-B (%)* Means ± SE	P‡	HOMA-S (%)† Means ± SE	P‡	BMI (kg/m^2)† Means ± SE	P§
SLC30A8													
rs13266634 (**C**/T)													
Genotype													
CC	960	5.64 ± 0.04		5.84 ± 0.03		77.0 ± 1.2		110.1 ± 1.4		67.8 ± 1.0		23.9 ± 0.1	
CT	1,411	5.57 ± 0.03	0.18	5.82 ± 0.02	0.61	80.3 ± 1.0	0.25	114.6 ± 1.1	0.05	65.6 ± 0.8	0.28	24.2 ± 0.1	0.0087
TT	531	5.56 ± 0.05		5.82 ± 0.04		78.6 ± 1.7		113.7 ± 1.8		66.5 ± 1.4		24.3 ± 0.2	
EXT2													
rs1113132 (**C**/G)													
Genotype													
CC	989	5.58 ± 0.04		5.82 ± 0.03		79.4 ± 1.2		112.3 ± 1.4		66.4 ± 1.0		24.1 ± 0.1	
CG	1,422	5.60 ± 0.03	0.97	5.83 ± 0.02	0.85	79.3 ± 1.0	0.26	114.0 ± 1.1	0.89	66.0 ± 0.8	0.37	24.1 ± 0.1	0.73
GG	506	5.57 ± 0.05		5.82 ± 0.04		76.7 ± 1.7		111.3 ± 1.9		68.4 ± 1.4		24.0 ± 0.2	
rs11037909 (**T**/C)													
Genotype													
TT	996	5.60 ± 0.04		5.82 ± 0.03		79.8 ± 1.2		112.4 ± 1.4		65.9 ± 1.0		24.2 ± 0.1	
TC	1,390	5.59 ± 0.03	0.73	5.83 ± 0.02	0.99	79.0 ± 1.0	0.20	114.0 ± 1.1	0.92	66.3 ± 0.8	0.23	24.1 ± 0.1	0.29
CC	509	5.58 ± 0.05		5.82 ± 0.04		77.1 ± 1.7		111.5 ± 1.9		68.1 ± 1.4		24.0 ± 0.2	
rs3740878 (**A**/G)													
Genotype													
TT	931	5.59 ± 0.04		5.81 ± 0.03		79.9 ± 1.3		113.0 ± 1.4		65.8 ± 1.0		24.2 ± 0.1	
TC	1,409	5.60 ± 0.03	0.83	5.83 ± 0.02	0.84	79.2 ± 1.0	0.18	113.4 ± 1.1	0.78	66.4 ± 0.8	0.24	24.0 ± 0.1	0.39
CC	518	5.57 ± 0.05		5.82 ± 0.04		76.9 ± 1.6		112.1 ± 1.9		67.9 ± 1.4		24.0 ± 0.2	
LOC387761													
rs7480010 (A/**G**)													
Genotype													
AA	1,718	5.59 ± 0.03		5.82 ± 0.02		79.9 ± 0.9		113.9 ± 1.0		65.7 ± 0.7		24.1 ± 0.1	
AG	1,034	5.56 ± 0.04	0.57	5.83 ± 0.03	0.97	78.3 ± 1.2	0.025	112.8 ± 1.3	0.08	67.1 ± 1.0	0.028	24.2 ± 0.1	0.87
GG	145	5.60 ± 0.10		5.82 ± 0.07		72.1 ± 2.9		106.1 ± 3.5		72.0 ± 2.8		23.8 ± 0.3	
Intergenic													
rs9300039 (**C**/A)													
Genotype													
CC	1,550	5.62 ± 0.03		5.84 ± 0.02		79.0 ± 1.0		112.5 ± 1.1		66.6 ± 0.8		24.1 ± 0.1	
CA	1,130	5.57 ± 0.04	0.07	5.81 ± 0.02	0.14	78.8 ± 1.1	0.99	112.9 ± 1.3	0.23	66.4 ± 0.9	0.87	24.1 ± 0.1	0.61
AA	212	5.46 ± 0.08		5.76 ± 0.06		79.2 ± 2.6		117.5 ± 3.0		66.2 ± 2.1		24.1 ± 0.2	

Data are *means ± SE or †geometric means ± SE, unless otherwise indicated. Alleles in bold are the risk alleles for type 2 diabetes identified by previous studies while alleles underlined are the risk alleles for type 2 diabetes or IFG observed in this study. ‡Adjusted for age, sex, region, and BMI. §Adjusted for age, sex, and region. ¶The associations remained significant after Bonferroni correction for multiple tests, and the Bonferroni corrected cutoff P value is 0.0014 (0.05/36 tests).

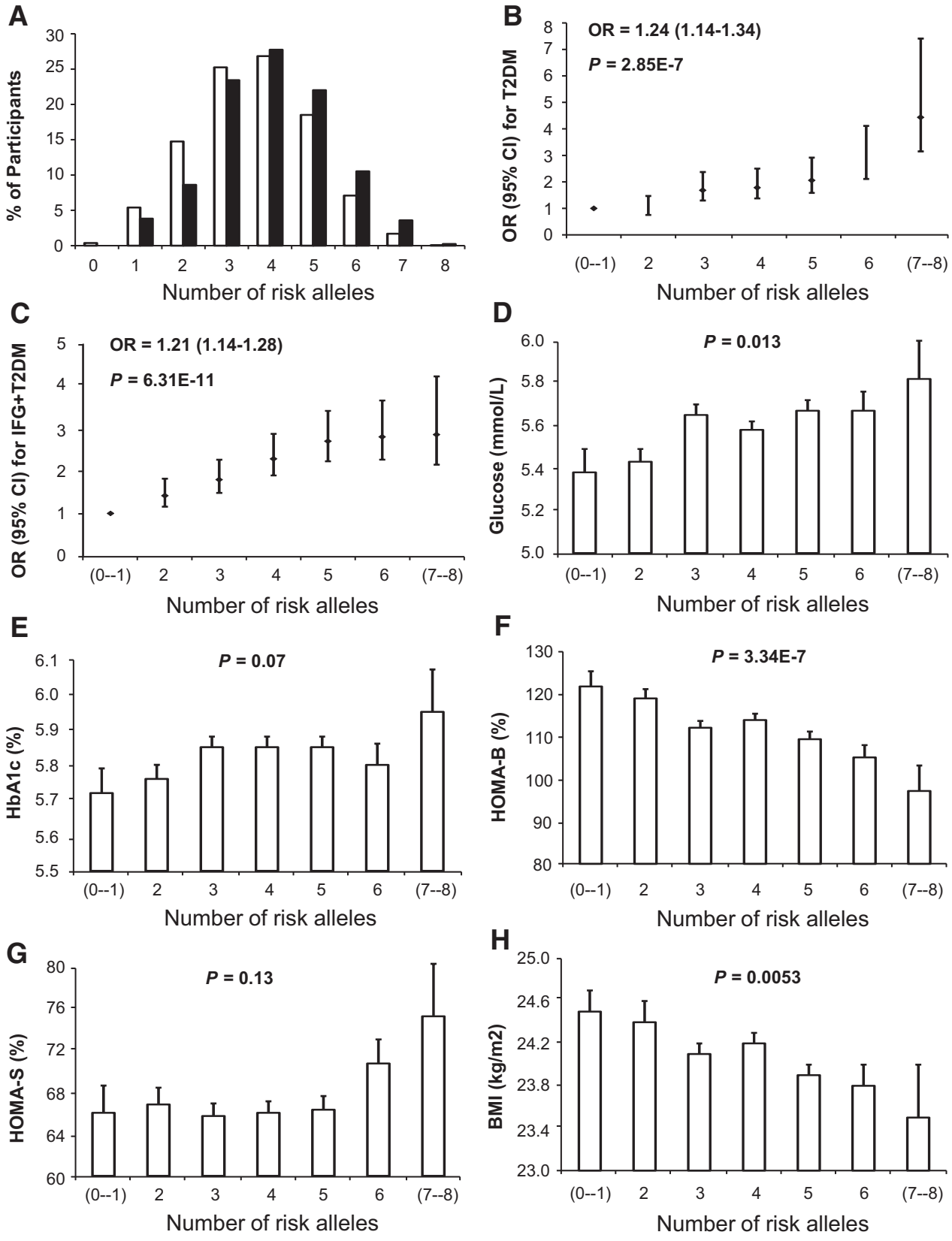

FIG. 1. Combined effects of increasing numbers of the risk alleles from *CDKAL1*-rs9465871, *CDKN2A/B*-rs10811661, *IGF2BP2*-rs4402960, and *SLC30A8*-rs13266634. *A*: The risk allele distribution in the participants with NFG and participants with type 2 diabetes. □, control; ■, type 2 diabetes. Each additional risk allele increased the risk of type 2 diabetes by 1.24-fold ($P = 2.85 \times 10^{-7}$) (*B*) and of IFG and diabetes combined by 1.21-fold ($P = 6.31 \times 10^{-11}$) (*C*). *B*: Participants harboring seven or all eight risk alleles had a 4.44-fold increased risk for type 2 diabetes ($P = 5 \times 10^{-4}$) compared with the reference group. Consistently, participants with increasing numbers of risk alleles tended to have increased fasting levels of plasma glucose ($P = 0.013$) (*D*) and A1C ($P = 0.07$) (*E*), as well as decreased HOMA-B values ($P = 3.34 \times 10^{-7}$) (*F*) and lower BMI ($P = 5.3 \times 10^{-3}$) (*H*), but showed no association with plasma insulin ($P = 0.13$) (*G*).

to detect previously reported odds ratios at $P < 0.05$. We did, however, find a significant association with combined IFG/type 2 diabetes. The associations with HOMA-B suggest that *IGF2BP2* confer type 2 diabetes risk through a reduced β-cell function. Similarly, we found no association between the *SLC30A8* rs13266634 variant and type 2 diabetes, while an association with combined IFG/type 2 diabetes reached borderline significance. Interestingly, the risk allele that increased diabetes risk in Europeans was also associated with a lower BMI in this population.

We also failed to find any evidence for association between type 2 diabetes and the SNPs in *EXT2* (rs3740878, rs11037909, and rs1113132) and the intergenic SNP rs9300039, despite ~80% power to detect previously reported effect estimates (7). Although these SNPs exhibited marginal associations with type 2 diabetes in the original study (7), they were largely negative in the subsequent four GWASs and other replication studies in samples from U.K. (8,12), Finnish (10,11), Swedish (10), Icelandic (9), German (27), and Japanese (23) populations. Therefore, the original associations for these SNPs were either population specific or overestimated due to the "winner's curse" (28,29), but the consistent lack of replication suggests that these findings were more likely false-positives. Meta-analyses or studies with larger sample sizes will be required to draw definitive conclusions. Although there was no association between rs7480010 (*LOC387761*) and type 2 diabetes or IFG, the allele conferring risk of diabetes in Europeans was associated with increased insulin sensitivity and showed a tendency toward a reduced β-cell function as well. For the three SNPs in *HHEX/IDE* gene region, the associations with type 2 diabetes or IFG were observed only in Shanghai individuals in whom each risk allele resulted in 1.45- to 1.79-fold increased diabetes risk, suggesting that geographical stratification may exist in our population for these SNPs and their roles in type 2 diabetes susceptibility. However, given the relatively small sample size, we cannot rule out sampling bias. This observation needs to be confirmed in larger studies.

We found no evidence of pairwise synergistic gene-gene interactions on type 2 diabetes and the related phenotypes among *CDKAL1*-rs9465871, *CDKN2A/B*-rs10811661, *IGF2BP2*-rs4402960, and *SLC30A8*-rs13266634. In joint analyses, the risk of type 2 diabetes was increased by 1.24-fold for each additional risk allele, and participants with seven or all eight risk alleles (3.8%) had a 4.44-fold increased risk of type 2 diabetes ($P = 5 \times 10^{-4}$) compared with those with one or no risk allele. These results are consistent with those reported by Scott et al. (11), who examined combined effects of 10 risk variants in a GWAS of European populations. Compared with Scott's study, the advantage of our study is that our data are based on the general population. However, a replication in larger population is required to examine whether combinations of risk alleles from these variants have good predictive and diagnostic potential in Chinese Hans.

In conclusion, we replicated the association of type 2 diabetes with the SNPs in *CDKAL1* and *CDKN2A/B* genes and confirmed that the SNPs in *SLC30A8* and *IGF2BP2* were associated with the risk of combined IFG/type 2 diabetes. Most of these SNPs were also associated with the impaired β-cell function. Importantly, the risk variants in *CDKAL1*, *CDKN2A/B*, *IGF2BP2*, and *SLC30A8* appear to act in an additive manner to increase the risk of type 2 diabetes and related phenotypes. These results provide solid evidence for the notion that these variants individually or collectively contribute to the risk of type 2 diabetes in the Chinese Han population, possibly by impairing β-cell function or reducing insulin secretion.

ACKNOWLEDGMENTS

This study was funded by research grants from the National Natural Science Foundation of China (30571562), a grant from the Knowledge Innovation Program Pilot Project of the Chinese Academy of Sciences (KSCX2-YW-R-73), the National Basic Research Program of China (973 program no. 2006CB503902), the Major Projects of Knowledge Innovation Program (KSCX2-YW-R-116 and KSCX1-YW-02), and the Shanghai-Unilever Research Development Fund (CH-2006-0941).

We are grateful to all participants of the Nutrition and Health of Aging Population in China. We also thank our colleagues at the laboratory and the local Centers for Disease Control and Prevention staff of Beijing and Shanghai for their assistance with data collection.

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