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SHORT COMMUNICATION

Polymorphism of *HMGA1* is associated with increased risk of type 2 diabetes among Chinese individuals

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Abstract
Aims/hypothesis Variants of the high-mobility group A1 (*HMGA1*) gene have been shown to be associated with insulin resistance and type 2 diabetes in individuals of European origin. We aimed to determine whether this locus confers significant susceptibility to type 2 diabetes in the Han Chinese population, and thus cross-race susceptibility to type 2 diabetes.
Methods Polymorphisms in *HMGA1* were identified by direct sequencing of genomic DNA derived from 192 Chinese participants (96 patients with type 2 diabetes and 96 controls). We then genotyped the common variant IVS5-13insC (c.136-14_136-13insC) in two other independent cohorts, including a total of 2,533 cases and 2,643 ethnically matched controls.
Results We confirmed the association of the *HMGA1* variant IVS5-13insC (c.136-14_136-13insC) with type 2 diabetes with an OR of 1.34 (95% CI 1.15, 1.56, p=0.0002 under a dominant model, and 95% CI 1.16, 1.55, p=0.0002 under an additive model) in the Han Chinese population, corresponding to a population attributable risk fraction of 5.0%.
Conclusions/interpretation *HMGA1* is an important susceptibility locus that confers a high cross-race risk of the development of type 2 diabetes.

Keywords Genetic association · Han Chinese population · *HMGA1* · Type 2 diabetes

Abbreviations

GWAS	Genome-wide association study
MAF	Minor allele frequency
PAR	Population attributable risk
SNP	Single-nucleotide polymorphism

Introduction

Insulin resistance has been well demonstrated to be a fundamental element in the aetiology of type 2 diabetes. There is considerable evidence that heredity is a major contributor to the insulin resistance found in type 2 diabetes [1]. The interaction of insulin with its target tissues is mediated by the insulin receptor (INSR), a glycoprotein that serves a crucial role in both directing insulin to its target cells and initiating the responses of these cells to the hormone [2]. The architectural transcription factor, high-mobility group A1 (HMGA1, OMIM 600701), has been implicated in the pathogenesis of type 2 diabetes via a regulation of *INSR* gene expression [3, 4].

Recently, Chiefari and colleagues demonstrated that individuals with type 2 diabetes had an increased prevalence over controls of the rare heterozygous variant IVS5-13insC (c.136-14_136-13insC) of the *HMGA1* gene [5], but the situation in the Han Chinese population still has to be established. In light of these novel findings, and considering that replication is a key criterion for establishing a convincing genetic association, we examined the association of this *HMGA1* variant with the risk of type 2 diabetes in the Han Chinese population.

L. Liu and H. Ding contributed equally to this study.

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Methods

Participants In the resequencing study, 96 participants with type 2 diabetes and 96 controls were recruited from individuals undergoing routine health examinations at Tongji Hospital in Wuhan (Hubei, People's Republic of China). The initial study involved 1,920 patients with diabetes and 1,920 controls. The patients were consecutively recruited from Tongji Hospital between September 2008 and December 2010. Controls matched ethnically, geographically and in terms of sex distribution were randomly selected from local community inhabitants participating in a community screening programme in Wuhan over the same period.

Type 2 diabetes was confirmed by OGTT according to the American Diabetes Association criteria [6] (n=992), by a report of the use of medication for diabetes (n=109) or based on a review of medical records (n=819). Type 1 diabetes was carefully excluded in our study on clinical grounds, from a review of medical records, on the basis of fasting C-peptide levels and from negative islet-related autoantibodies. The inclusion criteria for control participants were (1) that they were Han Chinese, (2) that there was no history of diabetes in their first- or second-degree relatives and (3) that they did not have a current diagnosis of diabetes.

To confirm the credibility of our results, we introduced a second independent case–control cohort comprising 613 cases and 723 controls. Cases were consecutively recruited from individuals attending the outpatient clinic of Tongji Hospital between January 2011 and July 2011 (n=547 by OGTT, and n=66 by reporting the use of medication for diabetes). The control participants, residing in the same communities as the cases, were determined to be free of type 2 diabetes by their medical history. The replication cohort followed the same inclusion criteria as the initial one. The study was approved by the institutional review board of Tongji Hospital. Written informed consent was obtained from all the participants. Experiments were conducted according to the principles expressed in the Declaration of Helsinki.

Single-nucleotide polymorphism identification and genotyping Fluorescent dye-terminator cycle sequencing was performed, and the products were analysed using an Applied Biosystems 3130xl capillary sequencer (Applied Biosystems, Foster City, CA, USA). Genotyping was performed with TaqMan single-nucleotide polymorphism (SNP) allelic discrimination by means of an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems) as previously described [7]. The probe for IVS5-13insC was ordered from Applied Biosystems (see electronic supplementary material [ESM] Table 1). Allelic discrimination was measured automatically using Sequence Detection Systems 2.1 software (autocaller confidence level 95%; Applied Biosystems, Foster City, CA, USA). The genotyping success rate was 98.9%, and the concordance rate was 100% based on 192 duplicates. No statistically significant deviations from Hardy–Weinberg equilibrium were observed (p>0.05).

Statistical analysis All statistical tests were performed using SPSS version 15.0 (SPSS, Chicago, IL, USA). The distribution of quantifiable variables was tested for normality using a one-sample Kolmogorov–Smirnov test. For comparison of the baseline characteristics between groups of participants, quantifiable variables were compared with one-way ANOVA. A χ^2 test was used to compare qualitative variables. The distributions of genotype in terms of variants were analysed for deviation from Hardy–Weinberg equilibrium using χ^2 analysis. Pairwise linkage disequilibrium coefficients between SNPs were calculated using Haploview software version 4.0 (Daly Lab at the Broad Institute, Cambridge, MA, USA) [8]. The general association of genotypes with type 2 diabetes was assessed by unconditional logistic regression analysis under dominant and additive genetic models with adjustment for covariates. Meta-analysis was carried out using Stata 10.0 software (STATA, College Station, TX, USA) and the Q statistic was calculated to test for heterogeneity. Population attributable risk (PAR) was calculated as $\mathrm{PAR} = (\chi-1)/\chi$. Assuming a multiplicative model, $\chi = (1-f)^2 + 2f\ (1-f)\gamma + f^2\gamma^2$ where γ is the estimated OR and f is the risk allele frequency.

Power to detect a genetic association was estimated using the QUANTO program version 1.2.3 (University of Southern California, Los Angeles, CA, USA). Assuming a minor allele frequency (MAF) of 0.067 and a disease prevalence of 5.5% [9], we achieved 97% and 98% power at p=0.05 to detect genetic effects at an OR of 1.34 under a dominant model and an additive model, respectively, in our combined samples. A p value <0.05 was considered statistically significant (two-tailed).

Results

The characteristics of the three cohorts are shown in Table 1. All the cases and controls from the three independent cohorts were self-reported as being of Han Chinese nationality and were carefully matched by specific geographical regions. The traditional risk factors for type 2 diabetes, such as hypertension, BMI and smoking status, were significantly different between the cases and the controls.

Resequencing of the *HMGA1* (NG_029020.1) gene identified 12 polymorphisms: six in promoter regions, four in introns, and two in the 3′ untranslated region (ESM Table 2). However, ten of these are rare and the other two SNPs (IVS5-13insC and rs35381162) within introns were common variants with the same frequency of 0.052 for a minor allele. Because IVS5-13insC is in strong linkage disequilibrium with rs35381162 (r^2=1.0), only IVS5-13insC was chosen for genotyping in the two additional independent cohorts, which

Table 1 Baseline characteristics of the study population

Variable	Sequenced study		Initial study		Replication study	
	Controls (n=96)	Cases (n=96)	Controls (n=1,920)	Cases (n=1,920)	Controls (n=723)	Cases (n=613)
Sex, male (%)	62 (64.6)	62 (64.6)	1,126 (58.6)	1,126 (58.6)	336 (46.5)	386 (63.0)*
Age (years)	50.56±8.57	49.89±7.27	56.84±9.61	53.23±12.44*	54.08±9.03	53.34±12.50
BMI (kg/m^2)	23.66±2.98	24.82±3.41*	24.42±3.27	24.72±3.68*	24.34±3.32	24.94±3.82*
WHR	0.90±0.06	0.90±0.06	0.87±0.07	0.91±0.06*	0.84±0.07	0.90±0.06*
SBP (mmHg)	124.97±14.57	126.43±15.87	126.21±19.41	134.02±23.15*	133.68±21.28	136.42±24.51*
DBP (mmHg)	79.26±8.63	81.64±10.11	77.86±10.68	83.61±13.53*	82.20±11.57	86.19±14.49*
HDL-C (mmol/l)	1.29±0.62	1.17±0.65	1.23±0.62	1.10±0.55*	1.45±0.33	1.09±0.31*
LDL-C (mmol/l)	2.35±0.89	2.59±0.97	2.43±0.89	2.51±0.95	2.85±0.78	2.52±0.95*
TC (mmol/l)	5.19±0.94	5.32±1.05	4.33±1.03	4.78±1.45*	4.82±0.94	4.08±1.48*
TG (mmol/l)	1.80±0.89	1.92±1.33	1.77±1.92	2.13±1.89*	1.28±0.93	1.68±1.20*
Hypertension (%)	22 (22.9)	21 (21.9)	604 (31.5)	777 (40.5)*	302 (41.8)	297 (48.5)*
Hyperlipidaemia (%)	26 (27.1)	33 (34.4)	414 (21.6)	613 (31.9)*	251 (34.7)	175 (35.4)
Smokers (%)	37 (38.5)	35 (36.5)	745 (38.8)	798 (41.6)*	195 (27.0)	240 (39.2)*

Data are means ± SD or percentages

Age for the case group refers to age at diagnosis; age for the control group refers to the age at which the participant was enrolled in the study

*p<0.05 vs control

C, cholesterol; DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglycerol

included 2,533 cases and 2,643 controls from the Han Chinese population, and the results from the independent resequencing study were subsequently combined (Table 2).

The MAF of the IVS5-13insC variant was higher among cases than among controls in the initial population (8.6% vs 6.6%). In the replication population, the MAF of the IVS5-13insC variant was 8.6% among cases vs 7.1% among controls. Meta-analysis showed significant genetic associations (OR=1.34, p=0.0002 under a dominant model, and OR=1.34, p=0.0002 under an additive model) under a random-effect model without evidence of heterogeneity (Q=0.37, p=0.833 and Q=0.36, p=0.836, respectively). Based on the estimated OR and risk allele frequency of IVS5-13insC from our meta-analysis, the PAR was 5%.

Table 2 Meta-analysis of the association between IVS5-13insC and type 2 diabetes among Han Chinese individuals

Population	Genotype, n (%)			Minor allele	Dominant model		Additive model	
	Del/del	Del/ins	Ins/ins		OR (95% CI)	$p_{dominant}$	OR (95% CI)	$p_{additive}$
Sequenced								
Control (n=96)	86 (89.6)	10 (10.4)	0 (0)	0.052				
Case (n=96)	81 (84.4)	15 (15.6)	0 (0)	0.078	1.95 (0.79, 4.83)	0.15	1.83 (0.77, 4.35)	0.17
Initial								
Control (n=1,920)	1,674 (87.2)	239 (12.4)	7 (0.4)	0.066				
Case (n=1,920)	1,600 (83.3)	310 (16.1)	10 (0.5)	0.086	1.35 (1.12, 1.63)	0.002	1.32 (1.11, 1.58)	0.002
Replication								
Control (n=723)	625 (86.4)	94 (13.0)	4 (0.5)	0.071				
Case (n=613)	512 (83.5)	97 (15.8)	4 (0.7)	0.086	1.28 (0.93, 1.75)	0.13	1.26 (0.94, 1.70)	0.12
Combined								
Control (n=2,739)	2,385 (87.1)	343 (12.5)	11 (0.4)	0.067				
Case (n=2,629)	2,193 (83.4)	422 (16.1)	14 (0.5)	0.086	1.34 (1.15, 1.56)	0.0002	1.34 (1.16, 1.55)	0.0002

$p_{dominant}$ and $p_{additive}$ significances for the adjusted OR (95% CI) were computed under dominant and additive genetic models with multivariate unconditional logistic regression analysis by adjusting for sex, age, BMI, hypertension, hyperlipidaemia and smoking status. Meta-analysis of three independent cohorts was combined by using a random effect model

Del, deletion allele; Ins, insertion allele

To explore whether covariates were modifying the observed associations, we conducted stratified analyses for case–control samples (ESM Table 3). The interactions were not statistically significant, indicating that potential interaction with or confounding by these factors had not meaningfully affected our results.

Discussion

Although our study aims to replicate the association between type 2 diabetes and *HMGA1*, it is the first of its kind to attempt to establish the association in individuals of non-European descent. Thus, our study emerges as a novel finding in an East Asian population, providing new evidence implicating the *HMGA1* locus as one conferring a high cross-race risk of development of type 2 diabetes. It is interesting to note that SNPs in *HMGA1* were rare in the European population [5]. However, IVS5-13insC was relatively common in Chinese people (MAF 8.6% and 6.7% in cases and controls, respectively) compared with individuals of white, European descent (MAF 3.7% and 0.9% in cases and controls, respectively); this may reflect the genetic heterogeneity between different ethnicities.

Recently, several genome-wide association studies (GWASs) of type 2 diabetes have been reported, but these did not detect an association between the *HMGA1* variant IVS5-13insC and the presence of type 2 diabetes. This new and interesting variant has not been registered by the NCBI, UCSC and HapMap databases, and current GWAS platforms, even Affymetrix Genome-Wide Human SNP Array 6.0 and Illumina Human1M-Duo BeadChip, do not contain the variant IVS5-13insC in their *HMGA1*; thus, current GWAS studies using commercial chips cannot detect the association. Given that type 2 diabetes is a complex disease, it is difficult to elucidate all the genetic risk factors from just a few GWAS studies, although GWASs are a powerful tool in the study of complex diseases.

In summary, and in accordance with previous evidence, a significant association between the *HMGA1* variant and type 2 diabetes was observed in our sample sets. Although the exact biological mechanism underlying the association between the *HMGA1* gene and risk of type 2 diabetes remains uncertain, these consistent findings indicate that *HMGA1* represents an important locus for predicting inherited susceptibility to type 2 diabetes. Additional studies are needed to investigate the impact of this gene on specific type 2 diabetes related-pathways and disease susceptibility in other racial groups.

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Contribution statement LL, HD, HRW, Y.JX, GLC, PHW and GY analysed the data and revised the manuscript; LL, HD, XFY and DWW were responsible for the conception of the study and drafting the article. All authors approved the final version.

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