GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genetic associations of the *INSIG2* rs7566605 polymorphism with obesity-related metabolic traits in Malaysian Malays

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Genet. Mol. Res. 13 (3): 4904-4910 (2014)
Received April 8, 2013
Accepted November 20, 2013
Published July 4, 2014
DOI http://dx.doi.org/10.4238/2014.July.4.4

ABSTRACT. A genome-wide association study showed that the tagging single nucleotide polymorphism (SNP) rs7566605 in the insulin-induced gene 2 (*INSIG2*) was associated with obesity. Attempts to replicate this result in different populations have produced inconsistent findings. We aimed to study the association between the rs7566605 SNP with obesity and other metabolic parameters in Malaysian Malays. Anthropometric and obesity-related metabolic parameters and DNA samples were collected. We genotyped the rs7566605 polymorphism in 672 subjects using real-time polymerase chain reaction. No significant associations were found between the rs7566605 tagging SNP of *INSIG2* with obesity or other metabolic parameters in the Malaysian Malay population. The *INSIG2* rs7566605 SNP may not play a role in the development

of obesity-related metabolic traits in Malaysian Malays.

Key words: Insulin-induced gene 2; Single nucleotide polymorphism; Obesity

INTRODUCTION

Obesity is a complex multifactorial disorder that occurs due to interactions between genetic and non-genetic factors (Walley et al., 2009; Russo et al., 2010; Fernandez et al., 2012). The World Health Organization (WHO) reported that approximately 2.8 million people die each year as a consequence of being overweight or obese (WHO, 2002).

Insulin-induced gene 2 (INSIG2) is a protein that mediates sterol regulation of sterol-regulatory element-binding proteins, cleavage-activating protein, and 3-hydroxy-3-methylglutaryl-coenzyme A reductase. INSIG2 plays important roles in cholesterol metabolism, lipogenesis, and glucose homeostasis (Goldstein and Brown, 1990; Yabe et al., 2002; Dong and Tang, 2010). Previous studies have shown that INSIG2 polymorphisms were associated with obesity, weight gain, and hypercholesterolemia. An *in vitro* analysis of allele-specific expression in human adipose tissue demonstrated that variants of the *INSIG2* gene were involved in body weight regulation in men and in the general population (Krapivner et al., 2008; Le Hellard et al., 2009).

A genome-wide association study reported an association between the rs7566605 variant in the upstream region of the *INSIG2* gene with body mass index (BMI) in the White American population, and this association was replicated in four cohorts (Herbert et al., 2006; Hotta et al., 2008; Zhang et al., 2008). On the other hand, many studies in other populations did not support this finding owing to the different genetic backgrounds of the studied populations (Hall et al., 2006; Feng et al., 2007; Kumar et al., 2007; Boes et al., 2008; Wang et al., 2008; Bressler et al., 2009; Deka et al., 2009; Wiedmann et al., 2009; Skelding et al., 2010). The ethnic composition of Malaysia is comprised of 52.4% Malays, 28.6% Chinese, 6.4% Indians, 10.8% Indigenous, and 1.8% other ethnic groups from different genetic pools. This study aimed to investigate associations between the *INSIG2* rs7566605 single nucleotide polymorphism (SNP) with various obesity, lipid, and cholesterol parameters in Malaysian Malays.

MATERIAL AND METHODS

Sample recruitment

Our study included 672 Malay participants from a public university in Kuala Lumpur, Malaysia, who were recruited through an annual voluntary health screening program, and from the Bera district of Pahang, Malaysia, who participated in a voluntary health screening. All participants were Malays for at least 3 generations as claimed by the self-report. This study was approved by the Medical Ethics Committee (MEC Ref. No. 672.23) of the University of Malaya Medical Center, and written informed consent was obtained from each participant. Subjects with a BMI above 30 kg/m^2 were categorized as obese, and those with a BMI below 30 kg/m^2 were categorized as non-obese.

Data collection

Ten to 15 mL overnight fasting blood samples were collected for routine biochemical measurements. Anthropometric measurements were performed to obtain data on height, weight, BMI, waist-hip-ratio (WHR), waist circumference (WC), and hip circumference (HC). The diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured. Waist and hip measurements were made by using a circumference measurement tape. HC was measured at the widest circumference over the buttocks and below the iliac crest, and WC was measured at the midpoint between the lower border of the rib cage (costal margin) and the iliac crest. Blood pressure was measured using a digital automatic blood pressure monitor (Omron HEM-907, Omron Healthcare, Kyoto, Japan).

DNA isolation

Genomic DNA was extracted from buccal swabs using the i-genomic CTB DNA extraction kit (iNtRON Biotechnology, Korea). The average optical density $(OD)_{260}/OD_{280}$ ratio was 1.88, which indicated good-quality DNA.

Genotyping

Genotyping of the *INSIG2* rs7566605 polymorphism was performed using the Taqman assay (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Genotypes of *INSIG2* rs7566605 were tested for deviation from Hardy-Weinberg equilibrium (HWE). The test for associations of the *INSIG2* rs7566605 polymorphism with obesity parameters was performed using generalized linear models. Data deviating from the normal distribution were log-transformed. The effects of SNPs on obesity parameters adjusted for age and gender were analyzed using a regression model at a significance level of $P < 0.05$. All results are reported as means ± standard deviation. The results of association analysis for the SNP and obesity parameters indicate the additive model that best fit the data. The additive model of the *INSIG2* rs7566605 polymorphism was entered as CC = 0, GC = 1, and GG = 2. Statistical analysis was performed using the SPSS 16.0 software. The power of the analysis was calculated using Quanto Version 1.2.4 (Menashe et al., 2008).

RESULTS

General characteristics of the 672 participants of this study are presented in Table 1. Five hundred participants were non-obese (BMI: 25.04 ± 3.01 kg/m^2), whereas 172 were obese (BMI: 33.47 ± 2.93 kg/m^2).

Table 2 shows the genotype and allele frequencies of the *INSIG2* rs7566605 SNP in the obese and non-obese participants. No significant differences were found between obese and non-obese groups for the allelic and genotype frequencies of the *INSIG2* rs7566605 SNP. Table 2 also shows that the *INSIG2* rs7566605 SNP did not deviate from HWE (non-obese HWE, P = 0.43; obese HWE, P = 0.69).

Table 1. General characteristics of the participants studied.

Parameter	Non-obese	Obese
Age (years)	46.47 ± 7.06 (N = 500)	47.97 ± 6.05 (N = 172)
Height (m)	1.60 ± 0.08 (N = 500)	1.59 ± 0.08 (N = 172)
Weight (kg)	64.58 ± 10.24 (N = 500)	84.40 ± 10.49 (N = 172)
BMI (kg/m^2)	25.04 ± 3.01 (N = 500)	33.47 ± 2.93 (N = 172)
WC (cm)	84.98 ± 9.30 (N = 500)	99.36 ± 8.45 (N = 172)
HC (cm)	98.65 ± 6.99 (N = 500)	112.45 ± 7.38 (N = 172)
WHR	0.86 ± 0.07 (N = 500)	0.89 ± 0.07 (N = 172)
SBP (mmHg)	127.28 ± 16.57 (N = 500)	136.57 ± 18.70 (N = 172)
DBP (mmHg)	79.68 ± 11.17 (N = 500)	87.82 ± 12.22 (N = 172)
TC (mM)	5.50 ± 0.94 (N = 347)	5.41 ± 0.95 (N = 117)
TG (mM)	1.39 ± 0.84 (N = 347)	1.54 ± 0.65 (N = 117)
LDL-C (mM)	3.55 ± 0.82 (N = 343)	3.46 ± 0.88 (N = 117)
HDL-C (mM)	1.32 ± 0.28 (N = 347)	1.25 ± 0.25 (N = 117)

BMI = body mass index; WC = waist circumference; HC = hip circumference; WHR = waist-hip-ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; TG = triglyceride; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.

Table 2. Allelic and genotypic distribution for *INSIG2* rs7566605.

Genotype/allele/HWE	Non-obese	Obese	P value
GG	0.32	0.33	0.90
GC	0.51	0.46	
CC	0.17	0.21	
G	0.43	0.44	0.75
C	0.57	0.56	
HWE (P value)	0.43	0.69	

HWE = Hardy-Weinberg equilibrium test.

Table 3 shows the genetic influence of the *INSIG2* rs7566605 SNP on obesity-related parameters. There was no significant difference in any of the obesity and obesity-related parameters between subjects with CC, GC, and GG genotypes after adjustment for age and gender. The CC homozygotes showed a trend toward higher weight, WC, SBP, and WHR compared to GC and GG carriers; the GG homozygotes showed a trend toward higher log-BMI, DBP, log-triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) compared to CC and CG carriers.

Table 3. Genetic effects of *INSIG2* rs7566605 SNP on obesity parameters.

Parameter	CC	GC	GG	P (R^2)
Height (m)	1.59 ± 0.09 (N = 213)	1.59 ± 0.09 (N = 336)	1.57 ± 0.10 (N = 123)	0.07 (0.51)
Weight (kg)	68.91 ± 13.48 (N = 213)	67.99 ± 14.56 (N = 336)	68.08 ± 13.64 (N = 123)	0.72 (0.07)
Log-BMI	3.29 ± 0.17 (N = 213)	3.27 ± 0.19 (N = 336)	3.30 ± 0.18 (N = 123)	0.50 (0.02)
WC (cm)	89.61 ± 11.49 (N = 213)	88.66 ± 11.57 (N = 336)	88.54 ± 12.92 (N = 123)	0.79 (0.05)
HC (cm)	102.07 ± 11.02 (N = 213)	101.40 ± 10.34 (N = 336)	102.40 ± 10.21 (N = 123)	0.66 (0.02)
WHR	0.91 ± 0.56 (N = 213)	0.87 ± 0.07 (N = 336)	0.87 ± 0.09 (N = 123)	0.53 (0.03)
SBP (mmHg)	130.93 ± 18.75 (N = 213)	130.19 ± 17.90 (N = 336)	130.75 ± 19.31 (N = 123)	0.96 (0.12)
DBP (mmHg)	82.04 ± 11.29 (N = 213)	81.96 ± 12.51 (N = 336)	82.43 ± 12.12 (N = 123)	0.90 (0.05)
Log-TG	0.20 ± 0.54 (N = 148)	0.23 ± 0.58 (N = 233)	0.25 ± 0.56 (N = 83)	0.59 (0.11)
TC (mM)	5.39 ± 0.91 (N = 148)	5.49 ± 1.01 (N = 233)	5.62 ± 0.81 (N = 83)	0.19 (0.02)
HDL-C (mM)	1.28 ± 0.25 (N = 148)	1.30 ± 0.29 (N = 233)	1.32 ± 0.24 (N = 83)	0.53 (0.15)
LDL-C (mM)	3.48 ± 0.79 (N = 148)	3.51 ± 0.92 (N = 233)	3.63 ± 0.67 (N = 83)	0.49 (0.01)

Means and P values were adjusted for age and gender. P values were obtained using generalized linear models. For abbreviations, see legend to Table 1.

DISCUSSION

There were no significant differences between the obese and non-obese groups in allelic and genotype frequencies of the *INSIG2* rs7566605 SNP. The genotype frequency of the CC homozygotes of the *INSIG2* rs7566605 SNP was higher in obese subjects compared to non-obese subjects in the Malaysian Malay population, as observed in previous studies (Goodman et al., 2005; Hotta et al., 2008; Wang et al., 2011). The International HapMap project revealed different frequencies of the C allele of the *INSIG2* rs7566605 polymorphism in different ethnic groups. In the present study, the frequency of the C allele was found to be 44% in the Malaysian Malay population. As a comparison, the frequencies of the C allele of the *INSIG2* rs7566605 polymorphism were 23% in the Uyghur population, 28% in the White American population, 36% in the Han Chinese and Japanese populations, 41% in the African population, and 37% or 31% in populations with Western European ancestry (Herbert et al., 2006).

This study showed no association between the *INSIG2* rs7566605 SNP with obesity. The lack of association found in the present study corroborates earlier findings from the Nurses' Health Study Cohort as well as another study in a Caucasian population (Hall et al., 2006; Herbert et al., 2006). A genome-wide association study (Herbert et al., 2006) found an association of the *INSIG2* rs7566605 polymorphism with obesity. Following this initial finding, replication of these findings was achieved in populations of Western European ancestry, African-Americans, and others (Herbert et al., 2006; Lyon et al., 2007; Hotta et al., 2008; Liu et al., 2008; Orkunoglu-Suer et al., 2008; Yang et al., 2008; Zhang et al., 2008). However, a few other studies carried out in French, European, German, Danish, British Caucasian, American-Samoan, and Mexican-American populations failed to show an association between the *INSIG2* rs7566605 SNP with obesity (Hall et al., 2006; Loos et al., 2007; Rosskopf et al., 2007; Andreasen et al., 2008; Bressler, et al., 2009; Deka, et al., 2009). Within the general Asian population, the association of the *INSIG2* rs7566605 SNP with obesity is inconsistent. A significant association between the *INSIG2* rs7566605 SNP with obesity was found in the Japanese population (Hotta et al., 2008), whereas a lack of association was found in the Chinese and Indian populations (Kumar et al., 2007; Wang et al., 2008). In this study, polymorphisms on *INSIG2* rs7566605 had no effect on obesity traits in Malaysian Malays. The *INSIG2* rs7566605 SNP was associated with BMI, but not with WHR, SBP, DBP, TG, and cholesterol levels in the Chinese minority group in Xinjiang Uyghur, Northwest China (Zhang et al., 2008). Polymorphisms on *INSIG2* rs7566605 were not associated with BMI, lipoprotein parameters, and free fatty acid levels in the Utah and Austrian populations (Boes et al., 2008). Similarly, the *INSIG2* rs7566605 polymorphism had no effect on TC, TG, HDL-C, LDL-C, or blood pressure parameters in the Chinese population (Feng et al., 2007). In addition, the *INSIG2* rs7566605 SNP had no effect on TG levels in two UK-based cohorts (Smith et al., 2007). No association was observed between this polymorphism and obesity-related traits, except for WHR, in White, Hispanic, and African-American subjects (Bressler et al., 2009). Similarly, there was no association between the *INSIG2* rs7566605 SNP and BMI or obesity-related traits in Indian subjects (Kumar et al., 2007).

In the present study, the *INSIG2* rs7566605 polymorphism was not found to be significantly associated with levels of TC, LDL-C, HDL-C, and TG in Malaysian Malays. This was similarly observed in Korean and Japanese populations (Hotta et al., 2008; Cha et al., 2009; Oki et al., 2009). The *INSIG2* rs7566605 SNP was not associated with BMI, WHR, plasma levels of cholesterols, or TG in the Slavonic Caucasian population (Hubacek et al., 2010).

To the best of our knowledge, this is the first study investigating the effects of the *INSIG2* rs7566605 SNP on obesity-related traits in Malaysian Malays. This study had sufficient power (96%) to detect a significant difference at the α significance level of 0.05. Since the participants were middle-aged and elderly individuals, these findings cannot be generally extrapolated to children in Malaysia. Results of this study also cannot be generalized to other ethnic groups within the Malaysian population such as Chinese, Indian, and others. Therefore, genetic association studies on *INSIG2* should be carried out in the future in other ethnic groups within the Malaysian population. In conclusion, our study provides evidence that the *INSIG2* rs7566605 SNP is not an important variant in predisposing Malaysian Malays to obesity.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We are grateful to the Wellness Program team of the University of Malaya, Malaysia, and to all the participants of this study. We would like to thank Professor Rosmawati Mohamed (University of Malaya), Ms. Devi Peramalah (University of Malaya), Sim Maw Shin (University of Malaya), and Shamsul Mohd Zain (University of Malaya) for their various contributions to this study. Research supported by funds from the University of Malaya (#RG075-09HTM) and from HIR-MOHE (#E000049-20001).

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