Association between GNB3 c.825C>T polymorphism and the risk of overweight and obesity: A meta-analysis

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ABSTRACT

Background: The association between G protein β-polypeptide 3 gene (GNB3) c.825C>T polymorphism (rs5443) and the risk of overweight/obesity has been investigated in many published studies, but the results were conflicting and inconclusive. A meta-analysis was performed to make a more accurate assessment of the relationship.
Methods: The PubMed, ProQuest Health & Medical Complete, Web of Science, Chinese Biomedical Medical databases (CBM), Chinese National Knowledge Infrastructure (CNKI), and Wan Fang databases were searched to identify eligible literatures. Pooled odds ratios (ORs) with the corresponding 95% confidence intervals (CIs) were used to assess the strength of association between GNB3 c.825C>T polymorphism and overweight/obesity.
Results: Eleven articles including 15 case–control studies with a total of 10,396 subjects (3171 cases of overweight/obesity and 7225 controls) were enrolled in the meta-analysis. The GNB3 c.825C>T was significantly associated with overweight/obesity under a recessive model (OR = 1.22, 95% CI: 1.04–1.44, P = 0.015). Moreover, the GNB3 825T allele was obviously associated with overweight alone in all inheritable models (P < 0.05) except in a recessive model (P = 0.084). In the stratification analysis by potential confounding variables, a significant association was observed between GNB3 c.825C>T polymorphism and overweight/obesity risk in males under an allelic model (P = 0.008), a homozygous model (P = 0.014), a recessive model (P = 0.005), and a dominant model (P = 0.049). And the results also showed that GNB3 c.825C>T polymorphism was significantly associated with overweight/obesity in subgroups of mean age less than 30 years, consistent with HWE, and high-quality studies (P = 0.027, P = 0.043, P = 0.040, respectively) under a recessive model, but not in other subgroups. Meta-regression also revealed that P value of HWE, publication year, and the quality scores of studies were the sources of heterogeneity in a recessive model and an allelic model. "Leave one out" sensitivity analyses indicated that the association was more significant after excluding some studies. The funnel plot and Egger's linear regression test and Begg's test revealed no apparent publication bias.
Conclusion: This meta-analysis suggests that the presence of TT homozygote might be one of the genetic factors susceptible to overweight/obesity and that males or aged under 30 years increase the genetic susceptibility.

1. Introduction

The prevalence of overweight and obesity has been rapidly increasing in the world. According to World Health Organization (WHO), more than 1.9 billion adults aged above 18 years and 42 million children under the age of 5 were overweight or obese globally in 2014. Obesity is characterized by abnormal or excessive body fat accumulation, which is a risk factor for type 2 diabetes, hypertension, cardiovascular diseases, cancers, and cognitive dysfunction (Mitchell et al., 2011). Therefore, obesity has become a major public health challenge. It is generally accepted that obesity is attributed to a shift in dietary and physical activity habits (El-Sayed Moustafa and Froguel, 2013). In addition to the unhealthy lifestyle, genetic factors are assumed to play an important role in obesity susceptibility, which hereditability has been estimated about 61%–80% (Nan et al., 2012). Numerous genes related to obesity have been found by candidate gene and genome-wide association approaches (Rao et al., 2014).

One commonly studied candidate gene for obesity is G protein β-polypeptide 3 gene (*GNB3*). Heterotrimeric G proteins are composed of an alpha, a beta, and a gamma subunit encoded by families of related genes, which are critical to translate signals from the cell surface into a cellular response in all cells of the body (Downes and Gautam, 1999). A study by Wang HY et al. showed a critical role in adipogenesis played by G proteins (Wang and Malbon, 1996). Elevation expression of Gi alpha 2 subunit or expression of constitutively active Gi alpha 2 promotes lipid accumulation and adipogenic conversion of cells (Su et al., 1993). In addition, transgenic mice lacking the Gi alpha 2 subunit are lean and deficient in fat mass (Moxham et al., 1993). G protein beta subunits are important regulators of alpha subunits and of certain signal transduction. A recent study by Goldlust IS et al. reported that the duplication of the *GNB3* gene has been linked to an obesity phenotype not only in humans but also in the transgenic mouse model (Goldlust et al., 2013). A single-nucleotide polymorphism (c.825C>T, rs5443) in exon 10 of *GNB3* has been reported that associated with the occurrence of a splice variant Gβ3s (Siffert et al., 1998). Despite a deletion of 41 amino acids and one WD repeat domain of the G beta subunit, splice variant Gβ3s is proved to be a biologically active protein and can ultimately enhance signal transduction via pertussis toxin-sensitive G proteins (Siffert et al., 1998; Rosskopf et al., 2000). *GNB3* c.825C>T polymorphism influences G protein receptor mediated signal transduction, including lipolysis. Functional studies have established that *GNB3* 825TT homozygote has lowered Gβ3 protein and impaired the function of adrenoceptors, thus reducing lipolysis in human fat cells (Rydén et al., 2002; Hauner et al., 2002).

The first study on the relationship between overweight/obesity and *GNB3* c.825C>T polymorphism was conducted in 1999 by Siffert W et al. They found a significant association between the 825T allele and overweight and obesity with odds ratios between 2 and 3 in Germans, Chinese, and black South Africans (Siffert et al., 1999). Since then, much research has focused on the association between *GNB3* c.825C>T polymorphism and overweight/obesity. However, the results were inconsistent and conflicting. Therefore, we performed a meta-analysis of previous studies to comprehensively evaluate the relationship between *GNB3* c.825C>T polymorphism and overweight/obesity susceptibility.

2. Methods

2.1. Search strategy

We searched the PubMed, ProQuest Health & Medical Complete, Web of Science, Chinese Biomedical Medical databases (CBM), Chinese National Knowledge Infrastructure (CNKI), and Wan Fang (Chinese) databases for all publications on the association between *GNB3* c.825C>T polymorphism and the risk of overweight and obesity, using the following search terms: ('G protein beta 3 polypeptide' or 'GNB3') and ('obesity' or 'obese' or 'overweight' or 'body mass index' or 'BMI') and ('polymorphism' or 'variant'). All studies were published from 1999, when the first study of the topic was reported, to 20 May, 2015. There were no language limitations on the search. "Related articles" option in PubMed was examined. Reference lists from the retrieved articles were also screened.

2.2. Inclusion and exclusion criteria

The inclusion criteria were as follows: 1) the study had a case control design for the association between the *GNB3* c.825C>T and the risk of overweight and obesity. 2) Complete information of allelic frequencies and genotypic frequencies was available in cases and controls for calculating the odds ratio (OR) with 95% CI directly and indirectly. 3) The criteria for diagnosis was established using body mass index (BMI) cut-off points for obesity. We have excluded studies for 1) review articles, case reports, editorials, or animal research, and 2) overlapping and insufficient data.

2.3. Quality assessment and data extraction

The quality of the studies was critically assessed using the Newcastle–Ottawa Quality Assessment Scale (NOS) by two authors (Li HL and Zhang YJ). The following aspects of each study were appraised: selection of cases and controls, comparability, and outcome or exposure. Quality scores ranged from 0 to 9. In addition, we assessed the quality of included studies by *P* value of Hardy–Weinberg equilibrium (HWE) for the control genotype. Studies only consistent with HWE were scored in part of "selection of control" (Table 2). The study that scored seven or more stars was considered as a high-quality study, otherwise, the low-quality study. Guidelines from meta-analysis of observational studies in epidemiology (MOOSE) group were followed to extract the following data: first author, publication year, country and ethnicity, source of control, sample size and mean age of cases and controls, genotyping method, *P* value for HWE, BMI cut-off points, number of gender (males/females), and the genotypic and allelic frequencies in cases and controls. The studies were reviewed by two authors respectively. The results were compared and disagreements were solved with consensus.

2.4. Statistical analysis

The STATA 12.0 software (StataCorp, College Station, TX, USA) was used for all statistical analyses. The association between *GNB3* c.825C>T polymorphism and overweight/obesity was assessed using crude odds ratio (OR) with 95% confidence interval (CI). The pooled ORs were determined for allelic model (T versus C), homozygous model (TT versus CC), heterozygous model (CT versus CC), recessive model (TT versus CT/CC), and dominant model (CT/TT versus CC). The pooled OR was calculated using the Z test with the significance set at $P < 0.05$. HWE was assessed using the Chi-square test. The heterogeneity between studies was evaluated using the I^2 test and Q statistic test. The value of I^2 lies between 0% and 100%, and the larger value indicates increasing heterogeneity (Higgins et al., 2003). If heterogeneity was observed between studies ($I^2 > 50\%$ or $P < 0.05$), the DerSimonian and Laird method for random-effects model was used to calculate the pooled OR and 95% CI. Otherwise, the Mantel–Haenszel method for fixed-effects model was adopted for the meta-analysis. Stratification analysis according to the *P* value of HWE, ethnicity, mean age, gender, and quality scores of studies were also performed to evaluate the association. Meta-regression was applied to explore the sources of between-study heterogeneity. The study publication year, ethnicity, BMI cut-off points, number of cases and controls, quality scores of studies and the *P* value of HWE were regarded as the potential confounding factors. Sensitivity analysis was performed to evaluate the effect of individual study on pooled results and assess the stability of results. The potential publication bias was graphically represented by funnel plots, and the funnel plot asymmetry was evaluated with Egger's linear regression test and Begg's test (Egger et al., 1997; Begg and Mazumdar, 1994).

3. Results

3.1. Study characteristics

A total of 780 studies were identified by searching databases. After carefully screening titles and abstracts, 26 articles were chosen after removing duplicates, animal research, and not significantly relevant papers. Finally, 11 articles including 15 case–control studies with a total of 3171 cases of overweight/obesity and 7225 controls were included after detailed evaluation in the present meta-analysis (Fig. 1). And the main characteristics of included studies are presented in Table 1. Two studies used polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) as genotyping methods (Lee et al., 2015; Hsiao et al., 2013), whereas thirteen studies used Taqman assay (Siffert et al., 1999; Wang et al., 2008; Chen et al., 2006; Zhou et al., 2005; Lee et al., 2005; Suwazono et al., 2004; Benjafield et al., 2001;
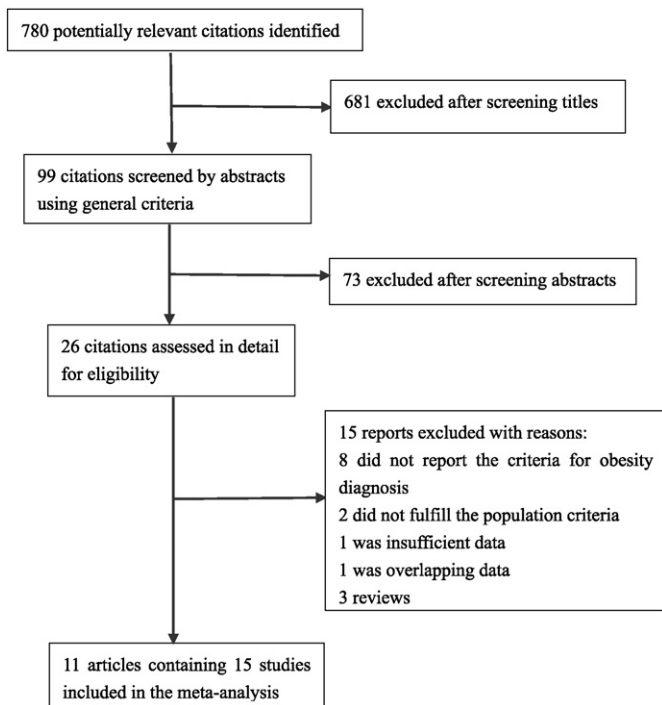
Fig. 1. Flow diagram of study selection.

Hinney et al., 2001; Ohshiro et al., 2001). All studies showed that source of controls was population-based except one (Hsiao et al., 2013). The quality of the studies was assessed by NOS scale and quality scores of the included studies were detailed in Table 2. Some included studies were separately analyzed to investigate the relationship between

GNB3 c.825C>T polymorphism and overweight or extremely obese alone. We also conducted a meta-analysis to estimate the association between GNB3 c.825C>T polymorphism and overweight or extremely obese respectively. The characteristics of studies of overweight or extremely obese and GNB3 c.825C>T polymorphism are showed in Table 3.

3.2. Meta-analysis

In the meta-analysis of all involved studies, there was a significant association between GNB3 c.825C>T polymorphism and overweight/obesity under an allelic model (OR = 1.08, 95% CI: 1.02–1.16, P = 0.017), a homozygous genetic model (OR = 1.19, 95% CI: 1.04–1.36, P = 0.014), as well as a recessive genetic model (OR = 1.19, 95% CI: 1.06–1.32, P = 0.002) (Table 4). But no significant association was observed under a heterozygous genetic model (OR = 1.00, 95% CI: 0.89–1.12, P = 0.994), and a dominant genetic model (OR = 1.05, 95% CI: 0.94–1.17, P = 0.350) (Table 4). Furthermore, the I^2 test and Q statistic test suggested an existence of heterogeneity in included studies under an allelic model, a homozygous model, a recessive model, and a dominant model. Therefore, the random effects model was used to calculate the pooled effects in those models. The results showed a significant association in a recessive model (OR = 1.22, 95% CI: 1.04–1.44, P = 0.015) between GNB3 c.825C>T polymorphism and overweight/obesity, but not in an allelic model (OR = 1.10, 95% CI: 0.99–1.22, P = 0.076) and in a homozygous genetic model (OR = 1.23, 95% CI: 0.98–1.24, P = 0.078) (Table 4).

Subgroup analysis was conducted in four models in which heterogeneity existed. In the stratified analyses using the P value of HWE, the results indicated only significant association in a recessive model consistent with HWE (OR = 1.15, 95% CI: 1.00–1.32, P = 0.043) (Table 5, and Fig. 2). Stratified analyses by ethnicity (categorized as Asian, Caucasian, and Black African) suggested only slightly significant

Table 1
Characteristics of studies of overweight/obesity and GNB3 c.825C>T polymorphism.

Author	Year	Country	Ethnicity	Source of control	Genotyping method	BMI cut-off points	Group	Number	Allele and genotypes					Mean age (y)	Gender (M/F)	P_{HWE}
									C	T	CC	CT	TT			
Lee et al.	2015	Korea	Asian	PB	TaqMan	85th	Case	394	379	409	85	209	100	8–9	231/163	0.023
							Control	1769	1746	1792	407	932	430	8–9	875/894	
Hsiao et al.	2013	China	Asian	HB	TaqMan	24	Case	467	402	532	87	228	152	About 40	333/134	0.188
							Control	505	441	569	89	263	153	40 ± 12	257/247	
Wang et al.	2008	China	Asian	PB	PCR–RFLP	25	Case	129	137	121	38	61	30	55 ± 10	85/44	0.495
							Control	270	285	255	78	129	63	54 ± 11	140/130	
Chen et al.	2006	China	Asian	PB	PCR–RFLP	23	Case	161	149	173	38	73	50	NA	58/103	0.001
							Control	313	358	268	88	182	43	NA	151/162	
Zhou et al.	2005	China	Asian	PB	PCR–RFLP	25	Case	142	154	130	43	68	31	>30	NA	0.584
							Control	85	83	87	19	45	21	>30	NA	
Lee et al.	2005	Korea	Asian	PB	PCR–RFLP	25	Case	130	132	128	32	68	30	25 ± 3	130/0	0.082
							Control	152	166	138	40	86	26	24 ± 3	152/0	
Suwazono et al.	2004	Japan	Asian	PB	PCR–RFLP	25	Case	183	195	171	52	91	40	42 ± 9	0/183	<0.001
							Control	989	1033	945	242	549	198	38 ± 9	0/989	
Suwazono et al.	2004	Japan	Asian	PB	PCR–RFLP	25	Case	322	322	322	80	162	80	40 ± 8	322/0	0.179
							Control	1131	1144	1118	278	588	265	38 ± 10	1131/0	
Benjafield et al.	2001	Britain	Caucasian	PB	PCR–RFLP	25	Case	84	122	46	42	38	4	48 ± 9	54/30	0.054
							Control	105	162	48	59	44	2	48 ± 10	55/50	
Hinney et al.	2001	Germany	Caucasian	PB	PCR–RFLP	90th	Case	491	695	287	251	193	47	About 20	225/296	0.957
							Control	110	144	76	47	50	13	25 ± 3	55/55	
Ohshiro et al.	2001	Japan	Asian	PB	PCR–RFLP	30	Case	263	272	254	71	130	62	About 50	146/117	0.872
							Control	150	148	152	37	74	39	49 ± 1	80/70	
Siffert et al.	1999	Germany	Caucasian	PB	PCR–RFLP	25	Case	92	108	76	32	44	16	About 20	92/0	0.753
							Control	207	292	122	102	88	17	About 20	207/0	
Siffert et al.	1999	China	Asian	PB	PCR–RFLP	25	Case	186	166	206	33	100	53	About 20	186/0	0.903
							Control	832	868	778	235	416	181	About 20	832/0	
Siffert et al.	1999	South Africa	Black African	PB	PCR–RFLP	25	Case	75	17	133	0	17	58	About 20	75/0	0.706
							Control	201	68	334	5	58	138	About 20	201/0	
Siffert et al.	1999	Zimbabwe	Black African	PB	PCR–RFLP	25	Case	52	17	87	1	15	36	About 20	52/0	0.753
							Control	406	151	661	15	121	270	About 20	406/0	

Abbreviations: BMI, body mass index; PB, population based; HB, hospital based; HWE, Hardy–Weinberg equilibrium.
P_{HWE} indicates the P value of Hardy–Weinberg equilibrium.

Table 2
Assessment study quality based on the Newcastle–Ottawa scale.

Author (year)	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-response rate	Total score
Lee et al. (2015)	★	★	☆[c]	★	★☆	★	★	☆	6
Hsiao et al. (2013)	★	★	★	★	★☆	★	★	☆	7
Wang et al. (2008)	★	★	★	★	★☆	★	★	☆	7
Chen et al. (2006)	★	★	☆	★	☆☆	★	★	☆	5
Zhou et al. (2005)	★	★	★	☆	☆☆	★	★	☆	5
Lee et al. (2005)	★	☆	★	★	★☆	★	★	☆	6
Suwazono et al. (2004)[a]	★	☆	★	☆	★☆	★	★	☆	5
Suwazono et al. (2004)[b]	★	☆	☆[c]	☆	★☆	★	★	☆	4
Benjafield et al. (2001)	★	★	★	★	★★	★	★	☆	8
Hinney et al. (2001)	★	★	★	★	★☆	★	★	★	8
Ohshiro et al. (2001)	★	☆	★	☆	★★	★	★	☆	6
Siffert et al. (1999)	★	★	★	★	★★	★	★	☆	8

[a] Indicates inconsistency with Hardy–Weinberg equilibrium.
[b] indicates that the subjects were female.
[c] Indicates that the subjects were male.

association in the recessive model (OR = 1.19, 95% CI: 1.00–1.43, P = 0.055) in the Asian subgroup (Table 5). In the subgroup analysis by mean age (categorized as mean age less than 30 years and mean age more than 30 years), we found only significant association in the recessive model (OR = 1.26, 95% CI: 1.04–1.55, P = 0.029) in the mean age less than 30 years subgroup (Table 5, and Fig. 3). Stratified analyses by gender (categorized as all, male and female) showed a significant association between GNB3 c.825C>T polymorphism and overweight/obesity in the male subgroup under an allelic model (OR = 1.27, 95% CI: 1.06–1.51, P = 0.008), a homozygous model (OR = 1.68, 95% CI: 1.11–2.54, P = 0.014), a recessive model (OR = 1.30, 95% CI: 1.08–1.56, P = 0.005) and a dominant model (OR = 1.38, 95% CI: 1.00–1.88, P = 0.049) (Table 5, and Fig. 4). In addition, we stratified the studies by quality scores,

and found that an obvious association was found in a high-quality study under the recessive model (OR = 1.30, 95% CI: 1.01–1.66, P = 0.040) (Table 5).

3.3. Heterogeneity analysis

The subgroup analysis revealed that heterogeneity was removed in the analysis of mean age above 30 years, and black African subgroup. Moreover, the heterogeneity consistent with the HWE subgroup was obviously removed with an I^2 value of 4.5% in the recessive model. In the allelic model, meta-regression showed that the P value of HWE, publication year, and study quality scores were the sources of heterogeneity between studies (P = 0.043, P = 0.055, P = 0.047, respectively), as well

Table 3
Characteristics of studies of overweight or extremely obese and GNB3 c.825C>T polymorphism.

Author	Year	Country	Ethnicity	Source of control	Genotyping method	BMI cut-off points	Group	Number	C	T	CC	CT	TT	Mean age (y)	Gender (M/F)	P_{HWE}
Overweight																
Hsiao et al.	2013	China	Asian	HB	TaqMan	24–27	Case	291	249	333	47	155	89	41 ± 1	204/87	0.188
							Control	505	505	441	569	89	263	40 ± 12	257/247	
Siffert et al.	1999	Germany	Caucasian	HB	PCR–RFLP	25–27	Case	70	85	55	26	33	11	24 ± 3	70/0	0.753
							Control	207	292	122	102	88	17		207/0	
Siffert et al.	1999	China	Asian	HB	PCR–RFLP	25–27	Case	128	118	138	24	70	34	25 ± 4	128/0	0.903
							Control	832	868	778	235	416	181		832/0	
Siffert et al.	1999	South Africa	Black African	HB	PCR–RFLP	25–27	Case	53	13	93	0	13	40	22 ± 4	53/0	0.706
							Control	201	68	334	5	58	138		201/0	
Chen et al.	2006	China	Asian	PB	PCR–RFLP	23–25	Case	57	58	56	12	34	11	NA	58/103	0.001
							Control	313	358	268	88	182	43		58/103	
Extremely obese																
Hinney et al.	2001	Germany	Caucasian	PB	PCR–RFLP	99th	Case	440	622	258	226	170	44	14 ± 3	199/241	0.957
							Control	110	144	76	47	50	13	25 ± 3	55/55	
Ohshiro et al.	2001	Japan	Asian	PB	PCR–RFLP	35	Case	55	57	53	13	31	11	50 ± 1	28/27	0.872
							Control	150	148	152	37	74	39	49 ± 1	80/70	

Abbreviations: BMI, body mass index; PB, population based; HB, hospital based; HWE, Hardy–Weinberg equilibrium.
P_{HWE} indicates the P value of Hardy–Weinberg equilibrium.

Table 4
Pooled ORs and 95% CIs of the association between GNB3 c.825C>T polymorphism and overweight/obesity in both fixed and random effect model.

Inherited model	Fixed effect model			Random effect model			I^2 (%)	P^H	Egger P	Begg P
	OR	95% CI	P	OR	95% CI	P				
T vs. C	1.08	1.02–1.16	0.017	1.10	0.99–1.22	0.076	53.3	0.008	0.412	0.520
TT vs. CC	1.19	1.04–1.36	0.014	1.23	0.98–1.54	0.078	54.0	0.007	0.296	0.656
CT vs. CC	1.00	0.89–1.12	0.994	1.00	0.87–1.14	0.945	16.9	0.264	0.454	0.299
TT vs. (CT + CC)	1.19	1.06–1.32	0.002	1.22	1.04–1.44	0.015	46.3	0.025	0.294	0.458
(TT + CT) vs. CC	1.05	0.94–1.17	0.350	1.06	0.91–1.22	0.484	38.6	0.063	0.381	0.458

P^H indicates the P value of heterogeneity.

Table 5
Pooled ORs and 95% CIs of subgroup analysis.

Subgroups	T vs. C			TT vs. CC			TT vs. (CT + CC)			(TT + CT) vs. CC		
	OR (95% CI)	P	I^2 (%)	OR (95% CI)	P	I^2 (%)	OR (95% CI)	P	I^2 (%)	OR (95% CI)	P	I^2 (%)
HWE												
Yes (n = 12)	1.09 (0.96–1.23)	0.184	49.7	1.18 (0.91–1.54)	0.213	47.3	1.15 (1.00–1.32)	0.043	4.5	1.07 (0.89–1.30)	0.504	45.3
No (n = 3)	1.14 (0.89–1.46)	0.289	75.0	1.37 (0.79–2.35)	0.259	78.5	1.46 (0.84–2.52)	0.178	85.6	1.03 (0.82–1.29)	0.815	25.3
Ethnicity												
Asian (n = 10)	1.08 (0.97–1.19)	0.174	48.9	1.18 (0.94–1.48)	0.165	56.1	1.19 (1.00–1.43)	0.055	54.2	1.03 (0.89–1.20)	0.689	31.9
Caucasian (n = 3)	1.18 (0.72–1.92)	0.517	80.6	1.64 (0.52–5.15)	0.399	76.4	1.50 (0.64–3.55)	0.355	0	1.16 (0.65–2.09)	0.605	75.6
Black African (n = 2)	1.36 (0.92–2.01)	0.129	0	2.65 (0.49–14.18)	0.256	0	1.33 (0.86–2.06)	0.202	62.6	2.52 (0.47–13.45)	0.278	0
Mean age												
Less than 30 (n = 7)	1.19 (0.99–1.44)	0.067	60.9	1.48 (0.99–2.21)	0.057	54.7	1.26 (1.04–1.55)	0.029	22.9	1.25 (0.91–1.70)	0.171	58.0
More than 30 (n = 7)	0.99 (0.90–1.09)	0.852	0	0.97 (0.80–1.17)	0.759	0	1.06 (0.91–1.23)	0.478	0	0.92 (0.80–1.08)	0.311	0
NA (n = 1)	1.55 (1.18–2.03)	0.001	–	2.69 (1.54–4.70)	0.000	–	2.83 (1.78–4.50)	0.000	–	1.27 (0.82–1.96)	0.293	–
Gender												
All (n = 8)	1.03 (0.89–1.18)	0.702	53.6	1.08 (0.80–1.46)	0.631	56.2	1.15 (0.87–1.51)	0.332	64.1	0.98 (0.85–1.12)	0.742	0
Male (n = 6)	1.27 (1.06–1.51)	0.008	44.8	1.68 (1.11–2.54)	0.014	46.9	1.31 (1.08–1.57)	0.005	0.9	1.38 (1.00–1.88)	0.049	45.3
Female (n = 1)	0.96 (0.77–1.20)	0.711	–	0.94 (0.60–1.48)	0.790	–	1.12 (0.76–1.64)	0.570	–	0.82 (0.57–1.16)	0.259	–
Study quality												
High (n = 8)	1.20 (0.97–1.50)	0.093	59.7	1.56 (0.94–2.60)	0.087	54.4	1.30 (1.01–1.66)	0.040	17.5	1.27 (0.89–1.82)	0.188	58.4
Low (n = 7)	1.05 (0.94–1.17)	0.388	41.8	1.11 (0.88–1.41)	0.372	49.7	1.19 (0.96–1.47)	0.118	61.0	0.98 (0.86–1.11)	0.730	0

n is the number of studies.

as in the homozygous model ($P = 0.013$, $P = 0.013$, $P = 0.013$, respectively). Furthermore, in the recessive model, meta-regression also revealed that the P value of HWE, publication year, and study quality scores were the sources of heterogeneity ($P = 0.008$, $P = 0.009$, $P = 0.021$, respectively), those three covariates were able to explain 86.47% of between-study variance, which was consistent with the subgroup analysis. In addition, meta-regression revealed that the study quality score was the source of heterogeneity in the dominant model ($P = 0.080$), which also was in agreement with the subgroup analysis.

3.4. Sensitivity analysis and publication bias

Sensitivity analysis was performed to evaluate the stability of results by excluding individual study each time. The analysis results
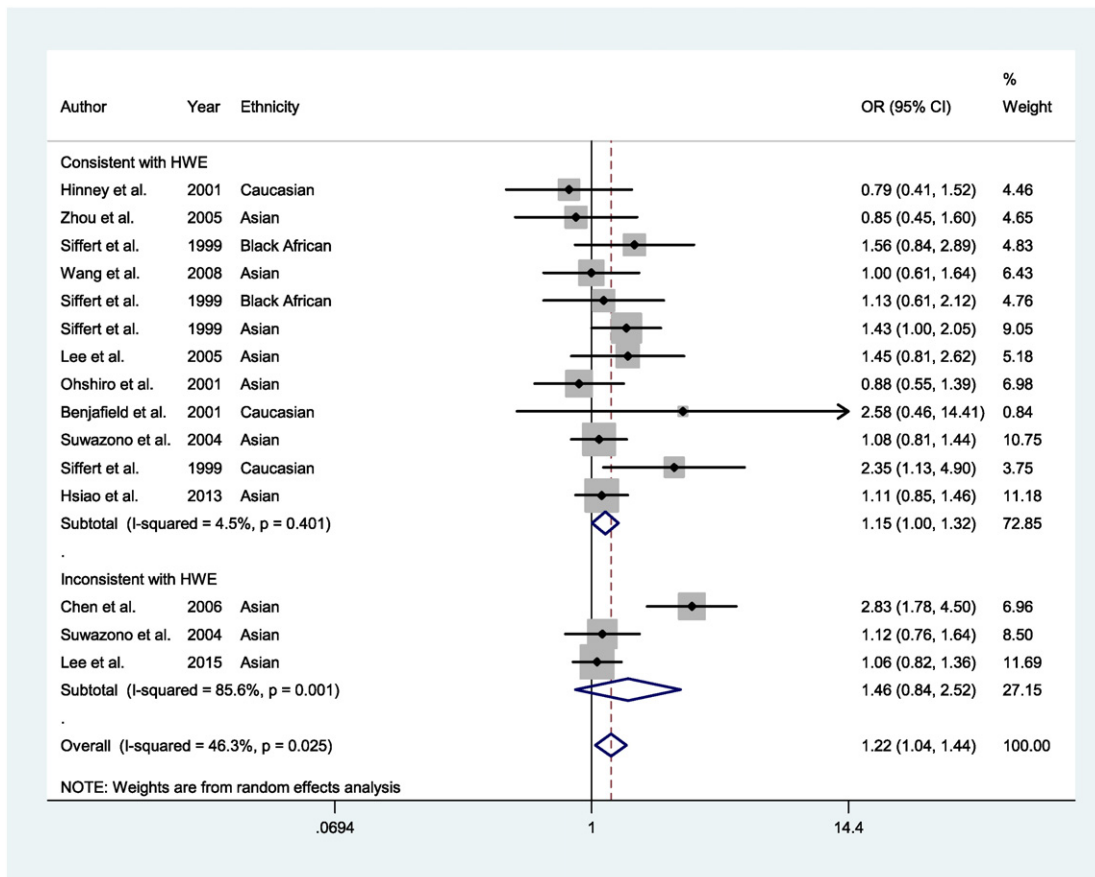

Fig. 2. Forest plot of overweight/obesity associated with GNB3 c.825C>T polymorphism in the recessive model (TT versus CT/CC) stratified by the P value of Hardy–Weinberg equilibrium.

demonstrated that the association was more significant after excluding the study by Hinney et al. under an allelic model (OR = 1.12, 95% CI: 1.01–1.24, $P = 0.027$), a homozygous model (OR = 1.28, 95% CI: 1.01–1.61, $P = 0.040$), and a recessive model (OR = 1.25, 95% CI: 1.06–1.47, $P = 0.009$), and the study by Zhou et al. under an allelic model (OR = 1.12, 95% CI: 1.00–1.24, $P = 0.041$), a homozygous model (OR = 1.27, 95% CI: 1.00–1.60 $P = 0.042$), and a recessive model (OR = 1.24, 95% CI: 1.05–1.47, $P = 0.010$). Moreover, the inter-study heterogeneity was completely removed after excluding the study by Chen et al. in recessive model. Deleting other individual study make little difference in corresponding pooled effects of all models (data not shown), which suggesting that our results are statistically robust. The funnel in all models revealed no obvious publication bias. Fig. 5 showed the funnel plots of the recessive model. The results were confirmed by Egger's linear regression test and Begg's test (Table 4).

3.5. Association between GNB3 c.825C>T polymorphism with overweight or extremely obese

Of 11 articles, five studies were separately analyzed the relationship between overweight and GNB3 c.825C>T polymorphism. We evaluated the relationship between overweight and GNB3 c.825C>T polymorphism alone. The results revealed a significant association between overweight and GNB3 c.825C>T polymorphism in all inherited models ($P < 0.05$) except a recessive model ($P = 0.084$) (Table 6). As noted, two studies were individually analyzed the association between extremely obese and GNB3 c.825C>T polymorphism. In contrast, no significant association was found in all inherited models ($P > 0.05$) between extremely obese and GNB3 c.825C>T polymorphism (Table 6).

4. Discussion

In the present meta-analysis with 11 eligible articles of total 10,396 subjects (3171 cases of overweight/obesity and 7225 controls), it is demonstrated that GNB3 825TT and the risk of overweight/obesity were significantly associated in recessive model, as well as overweight only in all models, but not in extremely obese. Further stratified analysis revealed that GNB3 825TT homozygote was a high risk factor of overweight/obesity in males or under age of 30 populations. To our knowledge, this is the first study to examine the subgroup analysis of mean age and potential sex differences in overweight/obesity with the association of GNB3 c.825C>T polymorphism.

Generally, it is considered that genes exert their effects across different ethnic groups. We performed a subgroup analysis by ethnicity, but no association was found under any inherited models. It is worth noting that the number of studies from Asian population was much more than for other races. And it is likely that the relationship between GNB3 c.825C>T polymorphism and overweight/obesity is more prevalent in ethnicities from Asia. Notoriously, overweight/obesity could be affected by age and sex. According to obesity epidemic in the United States, the overweight/obesity combined prevalence increase with age and more men than women were overweight/obesity, but there was no such trend regarding obesity (Wang and Beydoun, 2007). Thus, we performed a subgroup analysis in term of mean age and gender in present meta-analysis. There were significant associations between 825TT and overweight/obesity among those studies mean age under 30 years or males. In addition, the critical assessment of each original study quality is essential because the meta-analysis is a secondary analysis and evaluation of the original study. We adopted the NOS scale to assess the quality of each study. In subgroup analysis of study quality, we found
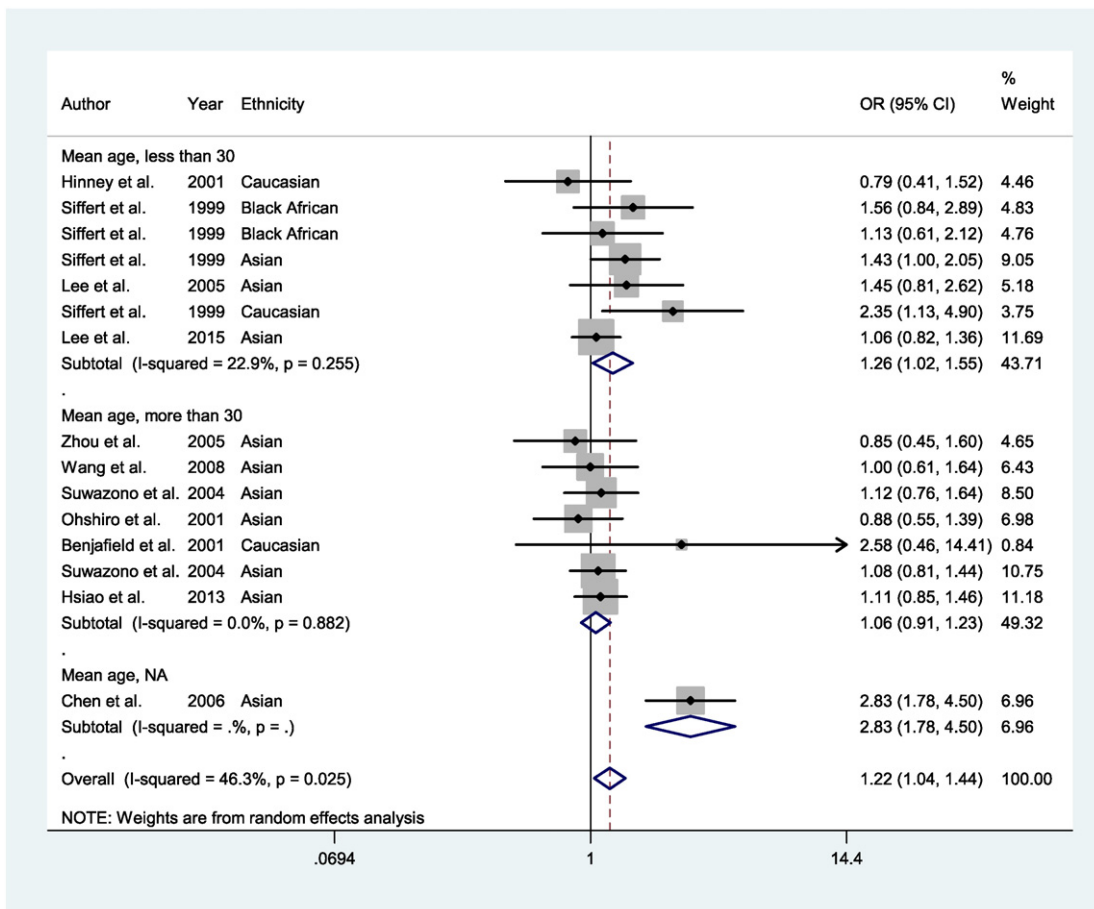

Fig. 3. Forest plot of overweight/obesity associated with GNB3 c.825C>T polymorphism in the recessive model (TT versus CT/CC) stratified by mean age.
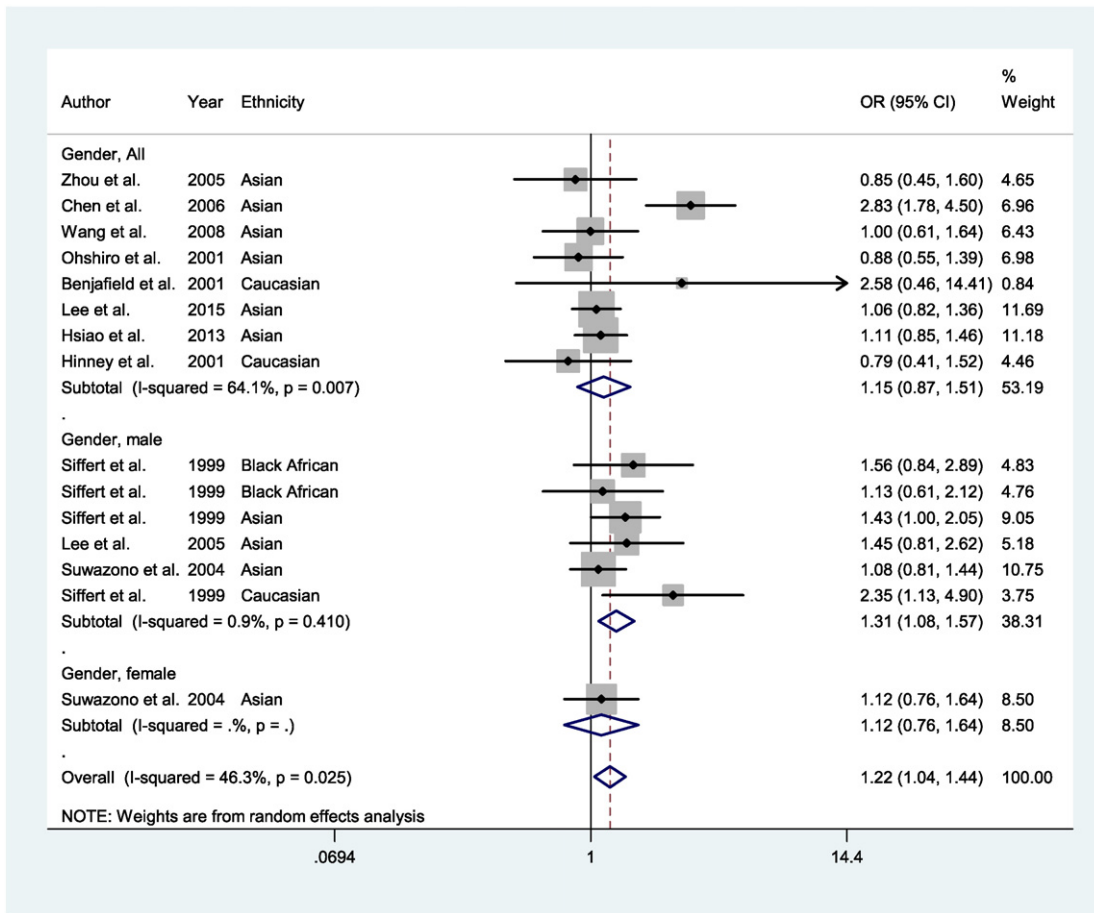
Fig. 4. Forest plot of overweight/obesity associated with GNB3 c.825C>T polymorphism in the recessive model (TT versus CT/CC) stratified by gender.

the pooled effect of high quality studies was closer to the overall pooled effect in all inherited model.

Currently, there are several different definitions for overweight and obesity. BMI is the most widely used measures, which provide a useful population-level measure of overweight and obesity. However, BMI cut-off points for overweight and obesity differ in different regions. And in children and adolescents, BMI 85th and 95th percentiles was defined as overweight (Must et al., 1991; Himes and Dietz, 1994). We separately

analyzed the association of the *GNB3* c.825C>T polymorphism with overweight or extremely obese alone and the results indicated the impact by the genetic factor of *GNB3* c.825C>T polymorphism was greater in overweight than extremely obese. The potential reasons maybe that the extremely obese could be affected more significantly than other factors or other genetic factors.

Heterogeneity between included studies is a common problem in meta-analysis. In present meta-analysis, moderate heterogeneity was found in four inherited models. To investigate this problem in greater depth, subgroup analysis and meta-regression analysis showed that quality scores of included studies were the sources of heterogeneity in the four inherited model. And the P value of HWE and publication year were the sources of heterogeneity in the allelic model, homozygous model, and recessive model. Furthermore, the funnel plot and Egger's linear regression test and Begg's test indicate no apparent publication bias in the present meta-analysis. "Leave one out" sensitivity analyses indicated that the association was more significant after excluding some studies.

Fig. 5. Funnel plots of overweight/obesity associated with GNB3 c.825C>T polymorphism in the recessive model (TT versus CT/CC).

Table 6
Pooled ORs and 95% CIs of the association between GNB3 c.825C>T polymorphism and overweight or extremely obese.

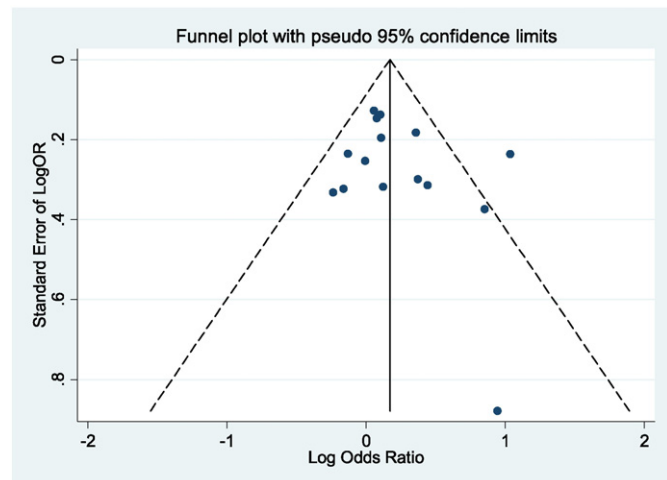| Inherited model	Overweight				Extremely obese			
	OR	95% CI	P	I^2 (%)	OR	95% CI	P	I^2 (%)
T vs. C	1.21	1.05–1.38	0.008	8.5	0.82	0.64–1.06	0.140	0
TT vs. CC	1.51	1.12–2.04	0.007	7.1	0.74	0.42–1.29	0.288	0
CT vs. CC	1.36	1.05–1.75	0.018	0	0.81	0.55–1.19	0.280	26.3
TT vs. (CT + CC)	1.21	0.98–1.51	0.084	0	0.77	0.47–1.27	0.307	0
(TT + CT) vs. CC	1.42	1.11–1.81	0.005	0	0.78	0.54–1.12	0.184	0

A previous meta-analysis by Souza et al. showed a trend ($P = 0.053$) associating CC and lower BMI under a fixed model (Souza et al., 2008). This suggests that the *GNB3* c.825C>T polymorphism was thought to play a key role in overweight/obesity. Our analysis confirmed the association of the *GNB3* c.825C>T polymorphism with overweight/obesity. More recently, a meta-analysis involving 10 studies by Tang et al. failed to observe association of *GNB3* c.825C>T polymorphism and overweight/obesity in a fixed effect model under a homozygous model (TT versus CC) (Tang et al., 2014). Their results were inconsistent with the present meta-analysis. The difference could be explained by that they included a review published in 2000 by Siffert et al. (2000), of which data overlapped with the study published in 1999 by Siffert et al. (1999). Moreover, they did not assess the association under other inherited models and perform the stratification analysis by the potential confounding variables. Several limitations in the current meta-analysis should be mentioned. Firstly, some included studies do not comply with HWE due to the fact that a very limited amount of studies reported the association between *GNB3* c.825C>T and the overweight/obesity, which may influence the cause–effect relationship. Fortunately, the overall pooled effects were in line with the subgroup analysis consistent with the *P* value of HWE, which illustrates that our results were stable. Secondly, we had insufficient information regarding genotypes and allelic frequency by different sex. In addition, some authors were contacted via email with respect to missing information, but data was not available.

In conclusion, our results suggested that the presence of *GNB3* 825TT homozygote might be one of the genetic factors susceptible to overweight/obesity and that male or aged less than 30 years increases the genetic susceptibility. We also demonstrated that *GNB3* 825TT and overweight alone were significantly associated. Additional large sample size and well-studied association studies are needed to provide powerful evidence to the conclusions.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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