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Original Article

Association of calpain-10 rs2975760 polymorphism with type 2 diabetes mellitus: a meta-analysis

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Abstract: Type 2 diabetes mellitus (T2DM) accounts for the majority of diabetes cases and affects a significant proportion of the adult population worldwide. Calpain-10 has been implicated in the development of type 2 diabetes, and some polymorphisms in the *CAPN10* gene have been associated with an increased risk of developing this disease. Several molecular epidemiological studies were conducted in recent years to evaluate the association between the *CAPN10* rs2975760 polymorphism and T2DM risk in diverse populations. However, the results remain conflicting rather than conclusive. We performed a meta-analysis of 8 case-control studies that included 2758 T2DM cases and 2762 case-free controls. We assessed the strength of the association, using odds ratios (ORs) with 95% confidence intervals (CIs). Overall, this meta-analysis showed that the *CAPN10* rs2975760 polymorphism was not associated with a significantly type 2 diabetes risk in three genetic models. However, after excluding two study for its heterogeneity, a significantly increased risk was found in all comparisons(for C *vs* T: OR=1.14, 95% CI=1.03-1.27, I^2=0, $P_{heterpgeneity}$=0.420, P_b=0.012; for TC *vs* TT: OR=1.15, 95% CI=1.01-1.30, I^2=3.8%, $P_{heterpgeneity}$=0.392, P_b=0.030; for CC+TC *vs* TT: OR=1.16, 95% CI=1.03-1.31, I^2=3.7%, $P_{heterpgeneity}$=0.393, P_b=0.015). No publication bias was found in the present study. This meta-analysis suggests that the C allele of the *CAPN10* rs2975760 polymorphism is associated with an increased T2DM risk. Further large and well-designed studies are needed to confirm this association.

Keywords: Type 2 diabetes mellitus, calpain-10, polymorphism, meta-analysis

Introduction

Diabetes mellitus is a heterogeneous group of metabolic diseases characterized by high blood glucose levels which, if untreated, lead to blindness, kidney and heart disease, stroke, loss of limbs and reduced life expectancy [1, 2]. It is a major public health problem affecting more than 347 million people worldwide [3]. Type 2 diabetes mellitus (T2DM) constitutes more than 90% of the cases of diabetes, and the prevalence has been dramatically increasing particularly in developing countries, such as China, which has 43.2 million diabetics, with 40-59-year-old patients being the largest age group impacted by the disease [4, 5].

T2DM is a multifactorial disease triggered by a combination of genetic and environmental risk factors. Calpains are ubiquitous calcium-activated proteases that are implicated in many cellular activities, including intracellular signal transduction, neuronal functions and cytoskeletal rearrangements [6]. The Calpain-10 (*CAPN-10*) gene was identified by Horikawa et al. [7] in Mexican Americans through a linkage scan, which identified polymorphisms that were associated with altered *CAPN10* expression. The highest expression of *CAPN10* mRNA is found in the heart followed by the pancreas, brain, liver, and kidneys [8]. Of particular interest, *CAPN10* is thought to be involved in glucose homeostasis as different calpain inhibitors have been shown to modulate insulin secretion and insulin action in rodents and human cell cultures [9, 10].

Variation in the *CAPN10* gene has been linked and associated with T2DM susceptibility. One polymorphism (UCSNP-43: G to A) and a specific haplotype combination defined by three polymorphisms (UCSNP-43, -19, and -63) were associated with a two- to threefold increased risk of T2DM in a sample of Mexican Americans and in two samples of European populations [7]. SNP44 (rs2975760), another intronic SNP,

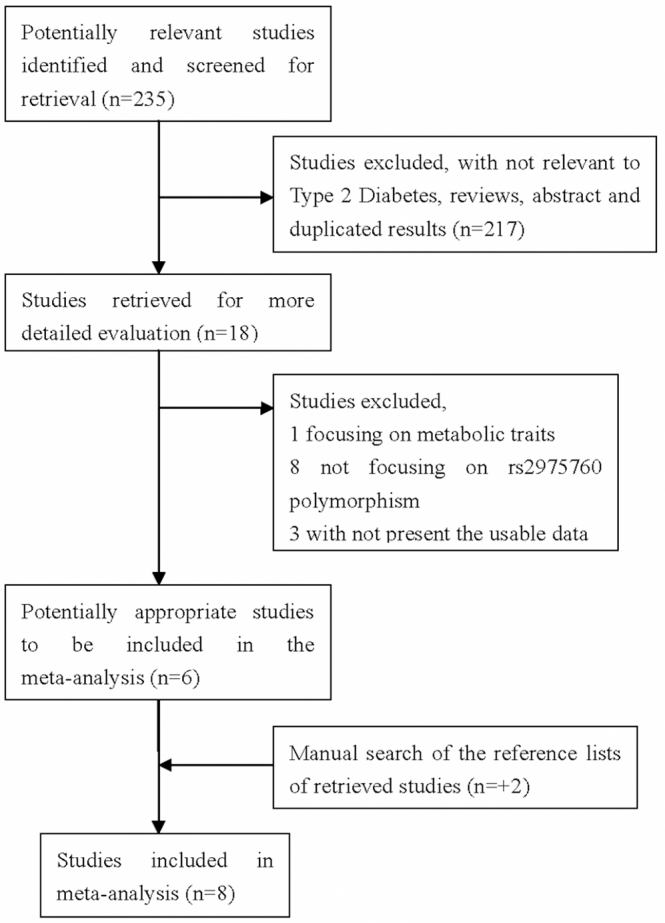

Figure 1. Flow chart of selection of studies and specific reasons for exclusion from the meta-analysis.

has also been associated with T2DM. Up to now, a few molecular epidemiological studies have investigated the association between the *CAPN10* rs2975760 polymorphism and T2DM risk [11-18]. However, the results remain controversial and ambiguous. Because a single study might have been underpowered to detect the overall effects, a quantitative synthesis of the accumulated data from different studies is important to provide evidence on the association of *CAPN10* rs2975760 polymorphism with T2DM risk. Hence, in the present study we conducted a meta-analysis to combine all studies available and validate whether the *CAPN10* rs2975760 polymorphism contributes to type 2 diabetes mellitus susceptibility.

Materials and methods

Publication search

We searched the PubMed and Embase databases for all articles on the association between *CAPN10* rs2975760 polymorphism and type 2 diabetes mellitus risk through May 2014. The following key words were used in this search: type 2 diabetes/T2D, polymorphism/variant, and Calpain10/*CAPN10*. The electronic searching was supplemented by checking reference lists from identified articles and reviews for additional original reports. The language of the reviewed articles was limited to English. All human-associated studies, regardless of sample size, were included if they All the studies must meet the following criteria: (1) case-control study; (2) the outcome had to be type 2 diabetes mellitus; (3) sufficient genotype data were presented to calculate the odds ratios(OR) with 95% confidence intervals (CI). The major exclusion criteria were: (1) no controls; (2) no sufficient data were reported. (3) Abstract, comment, review and editorial. Additionally, if more than one article was published using the same case series, we selected the study with the largest sample size.

Data extraction

All the available data were extracted from each study by two authors (S T Y and H T) independently according to the inclusion criteria listed above. Disagreement was resolved by discussion between the two authors. If these two authors could not reach a consensus, another author was consulted to resolve the dispute and a final decision was made by the majority of the votes. The following data were extracted: first author's name, year of publication, country of origin, ethnicity, definition of study patients (cases), genotyping method, total number of cases and controls, and genotype distributions in cases and controls.

Statistical analysis

The departure of frequencies of *CAPN10* rs2975760 polymorphism from expectation under Hardy-Weinberg equilibrium (HWE) was assessed by the chi-square test in controls and a $P < 0.05$ was considered as significant disequilibrium. The strength of the association between the *CAPN10* rs2975760 polymorphism and

Table 1. Characteristics of studies included in this meta-analysis

Author	Year	Country	Ethnicity	Genotyping methods	Sample size (case/control)	Case (%)			Control (%)			P_{HWE}
						TT	TC	CC	TT	TC	CC	
Laura	2004	USA	Mixed	PCR-RFLP	134/113	111 (82.8)	23 (17.2)	0 (0)	105 (55.3)	8 (40.4)	0 (4.3)	0.696
Einarsdottir	2006	Sweden	Caucasian	TaqMan	872/857	550 (63.1)	285 (32.7)	37 (4.2)	569 (66.4)	255 (29.8)	33 (3.9)	0.509
Chen	2007	China	Asian	PCR-RFLP	493/552	389 (78.9)	96 (19.5)	8 (1.6)	444 (80.4)	99 (17.9)	9 (1.6)	0.208
Demirci	2008	Turkey	Caucasian	PCR-RFLP	202/71	151 (74.8)	51 (25.2)	0 (0)	64 (90.1)	7 (9.9)	0 (0)	0.662
Bodhini	2011	India	Asian	PCR-RFLP	649/794	383 (59.0)	226 (34.8)	40 (6.2)	499 (62.8)	259 (32.6)	36 (4.5)	0.746
Andrea	2013	Mexico	Caucasian	TaqMan	40/32	33 (82.5)	7 (17.5)	0 (0)	24 (75.0)	8 (25.0)	0 (0)	0.419
Arslan	2014	Turkey	Caucasian	PCR-RFLP	118/93	85 (72.0)	33 (28.0)	0 (0)	56 (60.2)	36 (38.7)	1 (1.1)	0.066
Khan	2014	India	Asian	PCR-RFLP	250/250	156 (62.4)	83 (33.2)	11 (4.4)	161 (64.4)	80 (32.0)	9 (3.6)	0.808

PCR-RFLP: Polymerase Chain Reaction-restriction Fragment Length Polymorphism; HWE: Hardy-Weinberg Equilibrium.

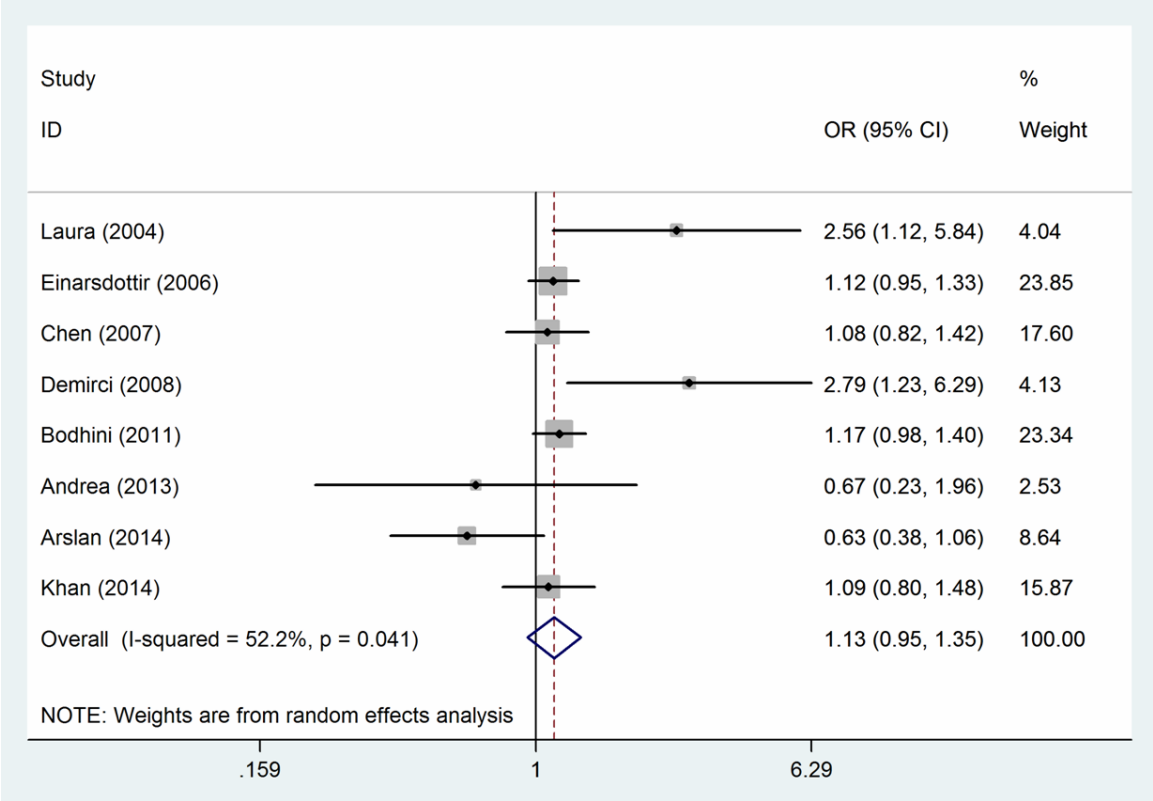

Figure 2. Odds ratios (OR) and 95% confidence interval (CI) of individual studies and pooled data for the association of *CAPN10* rs2975760 polymorphism and T2DM risk (C *vs* T). I^2, measure to quantify the degree of heterogeneity in meta-analyses.

type 2 diabetes risk was measured by odds ratios (ORs) with 95% confidence intervals (CIs). The significance of the pooled OR was determined by the Z-test, and $P < 0.05$ was considered as statistically significant. For *CAPN10* rs2975760, the meta-analysis examined the association between C allele and T2DM risk compared with that for T allele (C versus T); co-dominant model (TC versus TT) and dominant model (CC+TC versus TT) were also used. Subgroup analyses were done by ethnicity.

Heterogeneity among studies was checked by using the chi-square-based Q statistic and was considered statistically significant at $P < 0.10$ [19]. When $P > 0.10$, the pooled OR of each study was calculated by using the fixed-effects model (the Mantel-Haenszel method) [20]; otherwise, the random-effects model (the DerSimonian and Laird method) [21] was used. The Galbraith plot was used to detect the potential sources of heterogeneity, and re-analyses were conducted when the studies possibly causing the heterogeneity were excluded [22].

Publication bias was evaluated by visual inspection of symmetry of Begg's funnel plot and assessment of Egger's test [23] ($P < 0.05$ was regarded as representative of statistical significance). All analyses were done using STATA software, version 11.0 (STATA Corp., College Station, TX, USA), and all tests were two-sided.

Results

Characteristics of the studies

There were 235 papers relevant to the search words. The flow chart of selection of studies and reasons for exclusion is presented in **Figure 1**. Overall, 8 publications with 8 case-control studies including 2758 cases and 2762 controls were available for this analysis. Study characteristics are summarized in **Table 1**. Among those 8 studies, there were 4 studies about Caucasians, 3 studies about Asians and 1 study about mestizo, respectively. The genotype distributions among the controls of all studies were consistent with HWE (**Table 1**).

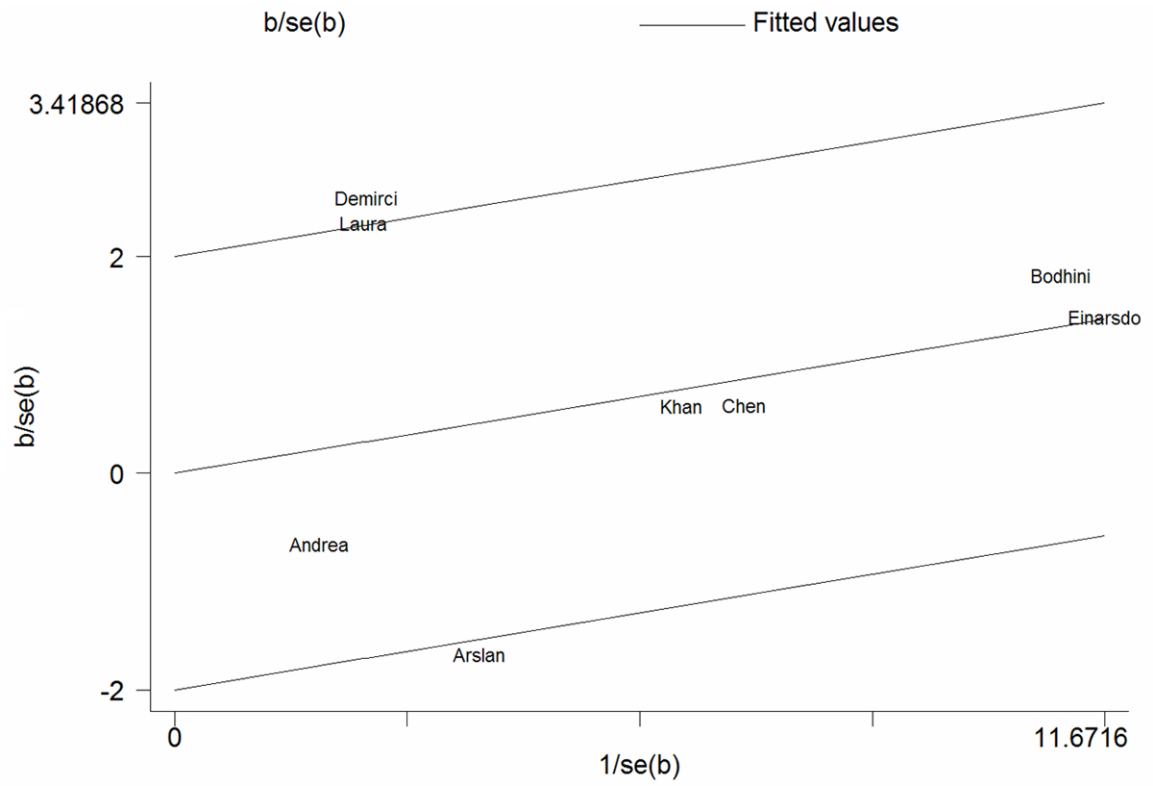

Figure 3. Galbraith plot of *CAPN10* rs2975760 polymorphism and T2DM risk. It indicated that two studies were the potential source of heterogeneity (Demirci et al. [14] and Arslan et al. [17]).

Quantitative synthesis

Overall, this meta-analysis showed that the *CAPN10* rs2975760 polymorphism was not associated with a significantly type 2 diabetes risk in these genetic models (for C *vs* T: OR=1.13, 95% CI=0.95-1.35, I^2=52.2%, $P_{heterpgeneity}$=0.041, P_b=0.175; for TC *vs* TT: OR=1.15, 95% CI=0.93-1.43, I^2=53.9%, $P_{heterpgeneity}$=0.034, P_b=0.200; for CC+TC *vs* TT: OR=1.16, 95% CI=0.94-1.43, I^2=55.4%, $P_{heterpgeneity}$=0.028, P_b=0.179) (**Figure 2**). Similarly, no associations were found in subgroup analysis based on ethnicity (data not showed). Heterogeneity between studies was observed in the overall comparisons as well as in subgroup analyses. To explorer the potential sources of heterogeneity further, we performed the Galbraith's test and accordingly singled out two study of Demirci *et al*. and Arslan *et al*. [14, 17] as the main contributors to heterogeneity (**Figure 3**). When excluding the two studies, the heterogeneity disappeared and a significantly increased risk was found in all comparisons (for C *vs* T: OR=1.14, 95% CI=1.03-1.27, I^2=0, $P_{heterpgeneity}$=0.420, P_b=0.012; for TC *vs* TT: OR=1.15, 95% CI=1.01-1.30, I^2=3.8%, $P_{heterpgeneity}$=0.392, P_b=0.030; for CC+TC *vs* TT: OR=1.16, 95% CI=1.03-1.31, I^2=3.7%, $P_{heterpgeneity}$=0.393, P_b=0.015) (**Figure 4**).

Publication bias

Begg's funnel plot and Egger's test were performed to assess publication bias among the literatures. No evidence of publication bias was observed in any comparison model (for C *vs* T, Begg's Test *P*=0.902, Egger's test *P*=0.749; for TC *vs* TT, Begg's Test *P*=0.536, Egger's test *P*=0.701; for CC+TC *vs* TT, Begg's Test *P*=0.902, Egger's test *P*=0.754) (**Figure 5**).

Discussion

Type 2 diabetes mellitus (T2DM), the most common form of diabetes, has alterations in insulin action and/or secretion [24]. Although numerous pathogenic processes are involved in its development, gene-environment interactions

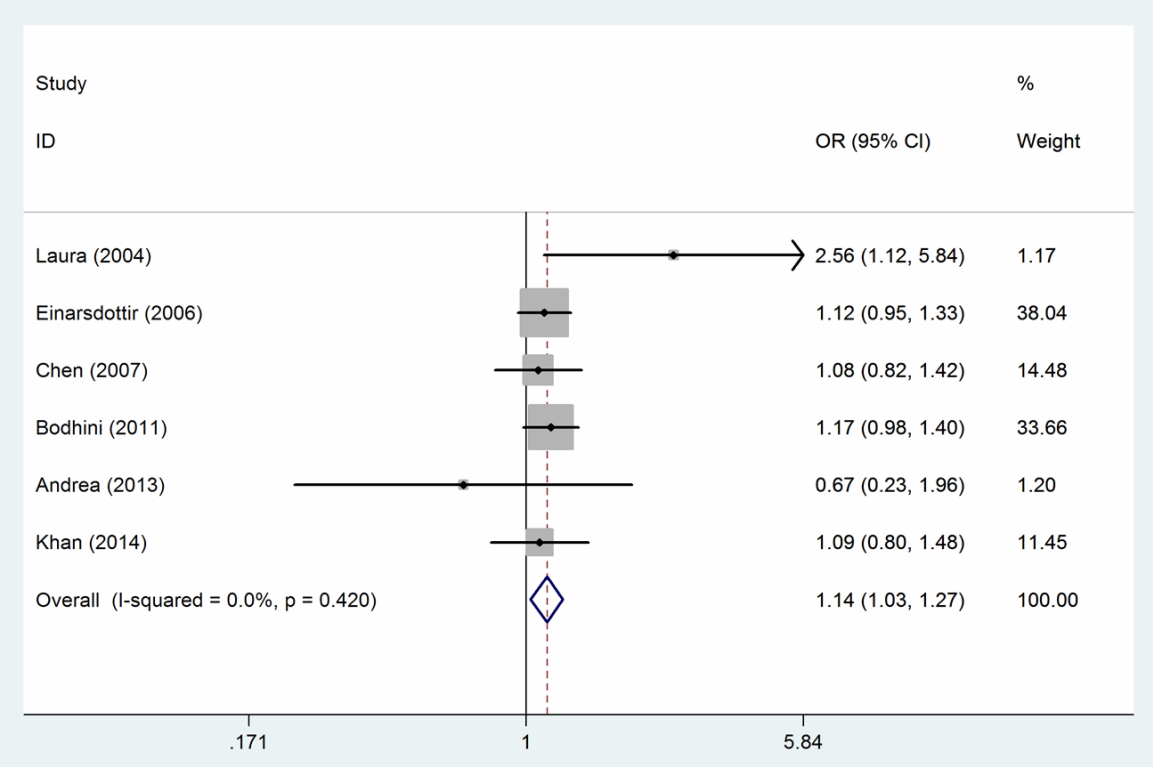

Figure 4. Forest plot showed the the association of *CAPN10* rs2975760 polymorphism and T2DM risk (C *vs* T).

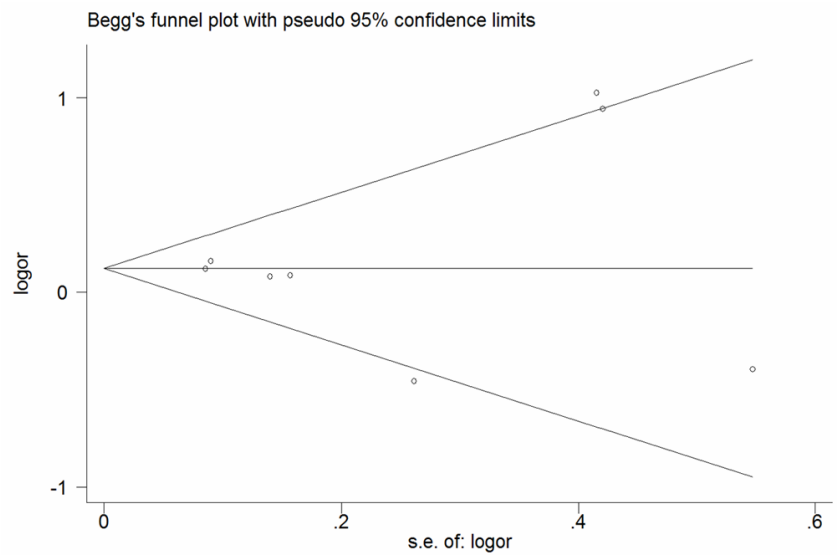

Figure 5. Begg's funnel plot for publication bias test (C *vs* T). Each point represents a separate study for the indicated association.

are essential for the development of T2DM. Thus, large efforts are focused to identify susceptibility genes in T2DM [25, 26]. Many genes have been associated with T2DM, such as *PPARG*, *KCNJ11*, *TCF7L2*, *CDKAL1*, *IGF2BP2*, *SLC30A8*, *HHEX*, *CDKN2A/B*, *KCNQ1* and *MTNR1B*, as determined through genome-wide association studies (GWAS) [27, 28]. *CAPN10* is an atypical member of the calpain family, which has a C2L (domain III) instead of a penta EF-hand domain [29]. *CAPN10* gene is located on chromosome 2q37.3, which has a region that was previously described as a susceptibility gene for diabetes, termed NIDDM1 (non-insulin dependent diabetes mellitus1). Genetic association studies and functional analyses have linked *CAPN10* to diabetes. Four main polymorphisms of *CAPN10* have been associated with diabetes: SNP-43 (rs3792267), SNP-44 (rs2975760), SNP-63 (rs5030952) and InDel-19 (rs3842570). These SNPs are localized in intronic regions and do not influence the amino

acid structure of the protein, but most likely alter the gene expression or alternative splicing mechanisms [7]. Recently, several studies have focused on the role of the rs2975760 polymorphism in T2DM [15-18]. However, the data reported for individual study were limited and not able to support a convincible conclusion. Therefore, in the current study, we performed a meta-analysis to evaluate the influence of SNP-44 in *CAPN10* on T2DM susceptibility.

In this meta-analysis, no association of the rs2975760 polymorphism with T2DM risk was found under all comparisons and in subgroup analysis by ethnicity. The significant heterogeneity was found among studies in overall comparisons and also subgroup analyses. To explorer the potential sources of heterogeneity further, we performed the Galbraith's test and accordingly singled out two study of Demirci et al. and Arslan et al. [14, 17] as the main contributors to heterogeneity. When excluding the two studies, the heterogeneity disappeared and a significantly increased risk was found in all comparisons. Therefore, our meta-analysis suggests that C allele of *CAPN10* rs2975760 polymorphism is associated with an increased T2DM risk.

As far as we know, this is the first comprehensive meta-analysis exploring the association between *CAPN10* rs2975760 polymorphism and T2DM risk up to now, which involved Caucasian, Asians and mixed European and Native American ancestry (mestizo) populations. Our meta-analysis also has some advantages. First, the genotype distributions among the controls of all studies were consistent with HWE. Second, the search and selection studies were conducted strictly. Third, when two studies were excluded from the analysis, the homogeneity of pooled studies was maintained, which guaranteed reliability of our analysis. Fourth, no evidence of publication bias was found by Begg's funnel plot and Egger's test, indicating that the whole pooled results may be unbiased.

Despite of the advantages mentioned above, the current study has some inevitable limitations that should be acknowledged. First, only published studies were included in this meta-analysis, unpublished data and ongoing studies were not sought, which may have biased our results. Second, there was significant heterogeneity among included studies. Even though we used the random-effect model to calculate pool ORs, the precision of outcome would be affected. Third, our results were based on an unadjusted estimated, a more precise analysis would have been conducted if more detailed individual data were available.

In conclusion, this metaanalysis suggests that the C allele of the *CAPN10* rs2975760 polymorphism is associated with an increased T2DM risk. However, rs2975760 is an intronic variant with comparatively lesser functional implications. Therefore, further studies screening the role of functionally relevant variants in the promoter and coding regions of *CAPN10* gene in linkage disequilibrium with rs2975760 are required to be undertaken on a larger sample size to establish the effect of *CAPN10* polymorphisms on the aetiology of the disease.

Disclosure of conflict of interest

None.

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