Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10S):187-192

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Association between SLC30A8 C/T (rs13266634) polymorphism and type 2 diabetes mellitus (T2DM): A meta-analysis of case-control studies

Thirunavukkarasu Ramasamy and Jayalakshmi Mariakuttikan*

Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Tamil Nadu, India

---

ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is the most common metabolic disease characterized by hyper-glycemia, due to impaired insulin secretion or action. One of the most studied genes is SLC30A8 (ZnT8) which is expressed majorly in pancreatic β-cells and participates in storage and transport of insulin. To analyze the association between Zn^+ transporter 8 gene (SLC30A8 rs 13266634) polymorphism and T2DM. In this study a systematic electronic search in PubMed, Google Scholar, Embase, Science Direct and Web of Science was carried out by two investigators. Pooled odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated to assess the strength of the association by using MedCalc® 15.4 software. Based on the inclusion and exclusion criteria, fourteen studies belonging to the Asian population involving 9232 T2DM patients and 8384 controls were considered in the Meta-analysis. The combined overall analysis revealed that the CC genotype of SLC30A8 C/T polymorphism exhibited a significant association with T2DM (Dominant model: OR=0.858; 95% CI=0.792-0.929; P<0.001) explaining the risk of C allele with diabetes. In the subgroup dominant model frequency comparison also the results indicated that rs13266634 C/T polymorphism was significantly associated with increased T2DM risk among E. Asians (OR = 0.612, 95% CI: 0.539 –0.6996, P < 0.001). Similarly, the Additive model revealed association of C/T with T2DM risk in S. Asians (OR = 0.967, 95% CI: 0.799 –1.17, P < 0.047). The present meta-analysis result indicates that C allele of SLC30A8 C/T polymorphism is associated with risk of T2DM in the Asian continent. However, larger prospective studies are needed to validate such meta-analysis.

Keywords: ZnT8 gene; Type 2 Diabetes mellitus; Genetic Polymorphism； SLC30A8rs13266634; Meta-analysis.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex disease characterized by insulin resistance and pancreatic β-cell dysfunction. Environmental factors combined with multiple genetic variants are involved in the onset and development of type 2 diabetes. To date, intense research has been performed to identify the genetic risk factors of type 2 diabetes and 18 genetic loci have been reported to be associated with the risk (1, 2). Overwhelming evidences indicate that there is a significant relationship between zinc transporters and diabetes mellitus. The solute carrier family 30 member 8 (SLC30A8), a novel member of the zinc transporters, maps to chromosome 8q24.11, contains eight exons, and encodes a 369 amino acid protein, the zinc transporter-8 (ZnT8). This transporter is localized in insulin secretory granules, and plays a major role in transport of the zinc from the cytoplasm to intracellular insulin containing vesicles for insulin maturation, storage and secretion (3, 4). The C to T single nucleotide polymorphism (SNP) rs1326634 of SLC30A8 gene results in a non- synonymous mutation changing from arginine (R) to tryptophan (W) at position 325.Incidence of arginine affects the normal function of ZnT8 further leading to β-cell destruction (5). The rs1326634 C/T polymorphism has been reported with higher risk of T2DM in various studies which includes five independent human genome-wide association studies (6). In the present study, we analyzed the results of all-inclusive search of literature and meta-analysis to explore the role of SLC30A8 gene (rs1326634) polymorphism in T2DM.

EXPERIMENTAL SECTION

Search strategy

This study carried out a systematic electronic search in PubMed, Google Scholar, Embase, Science Direct and Web of Science by two investigators. We focused on the most-studied rs13266634 C/T polymorphism, and identified all the relevant papers published in English, using the key words, case-control study of Type 2 diabetes; polymorphism of SLC30A8; polymorphism of ZnT8; rs13266634 in combination with T2DM. We included only those studies which were carried out from the geographical area of the Asian continent. Published manuscripts from electronic databases were retrieved and analyzed. The inclusion criteria were as follows: (1) original studies in case–control study design; (2) the number of subjects reported with each allele or genotype in cases and controls; (3) sufficient published data for estimating an odds ratio (OR) with 95% confidence interval (CI) or provided raw data that allowed us to calculate them. Animal studies, case reports, review articles, abstracts, editorials, reports with incomplete data, and studies based on pedigree data were excluded.

Data extraction

Two investigators independently extracted data using a standard form and reached consensus. The following data were collected from each study: the first author's name, publication date, ethnicity, country, sample size, genotyping method, allele frequency and odds ratio. Based on inclusion and exclusion criteria, fourteen studies from Asian population involving 9232 T2DM patients and 8384 controls were considered for the Meta-analysis.

Statistical analysis

The statistical analysis was performed using MedCalc® 15.4 software. The association between SLC30A8 (rs13266634) C/T polymorphism and risk of T2DM was interpreted by calculating OR with 95% CI that were used to assess the strength of association in the meta-analysis. The significance of the pooled OR was determined by the Z-test, and $P < 0.05$ was considered statistically significant. The allelic contrast (C versus T, allelic model) and the following genotype contrasts: homozygotes CC versus homozygotes TT (CC versus TT, additive model); homozygotes CC versus a combination of CT and TT (CC versus CT + TT, dominant model) and a combination of CC and CT versus TT (CC + CT versus TT, recessive model) were analyzed. Forest plot was selected for representation of the data. The Q test was used to measure the weighted sum of squares on a standardized scale to assess the heterogeneity between the data. Further I^2 value was calculated which denotes the percentage of observed total variation across the studies that are due to real heterogeneity rather than chance. A value of zero percent indicates no observed heterogeneity, and larger values show increasing heterogeneity. The Begg's funnel plot was used to test the publication bias among the included studies.

Figure 1: Flow chart of the study selection

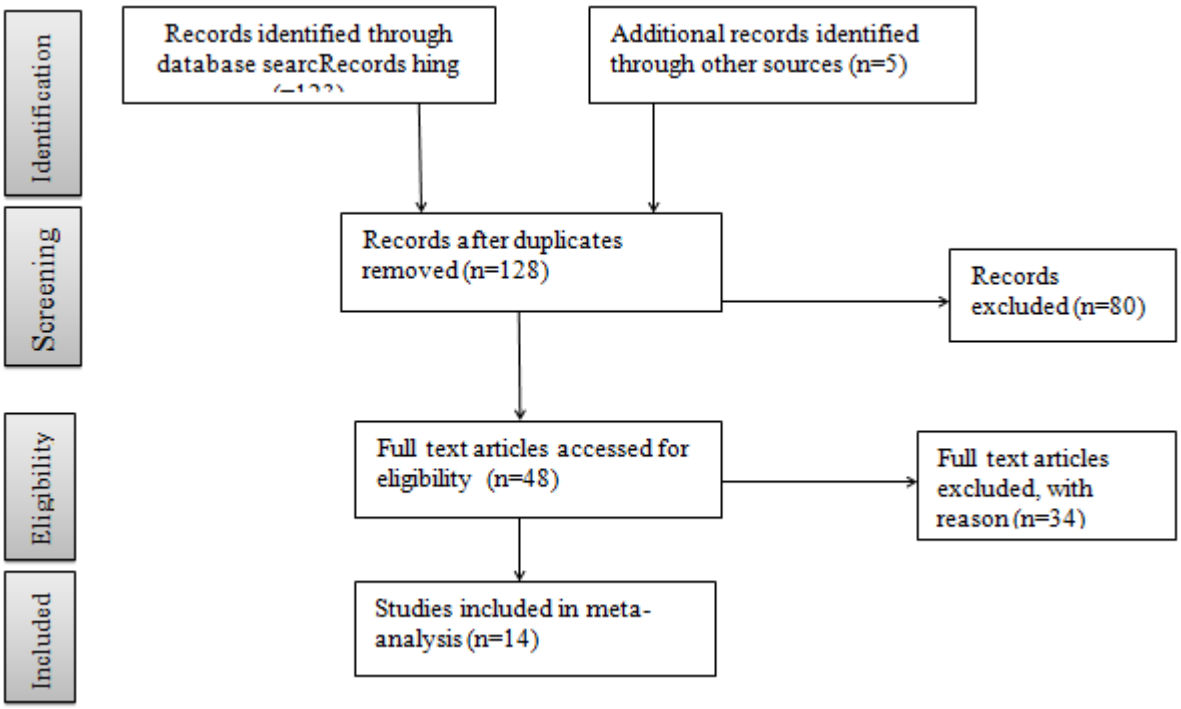


Table: 1 Characteristics of the individual studies and distribution of SLC30A8 C/T (rs 13266634) genotype and allele among T2DM patients and controls, included for meta-analysis

Study	Arms	C	T	CC	CT	TT	HWE	Population
Horikoshi et al.(2007)	case (n= 860)	681	1039	149	383	328		
	control (n= 859)	738	980	172	394	293	0.06	Japanese
Ng et al. (2007)	case (n= 433)	940	26	408	24	1		
	control (n= 419)	818	20	399	20	0	0.617	Hong Kong
Chang et al.(2007)	case (n= 760)	1483	37	724	35	1		
	control (n= 760)	1476	44	716	44	0	0.411	Taiwan
Sanghera et al.(2008)	case (n=532)	775	289	290	195	47		
	control (n= 386)	511	187	188	135	26	0.795	N. Indian
Lee et al.(2008)	case (n=908)	1107	709	324	459	125		
	control (n= 207)	560	444	156	248	98	0.974	Korean
Ren et al. (2008)	case (n=481)	917	45	438	41	2		
	control (n= 207)	952	30	463	26	2	0.0189	Beijing
Rong et al. (2009)	case (n= 1381)	2521	241	1156	209	16		
	control (n= 1766)	3206	326	1455	296	15	0.989	Pima Indian
Potapov et al.(2010)	case (n=588)	931	245	350	231	7		
	control (n= 597)	880	314	318	244	35	0.184	Russian
Lin et al. (2010)	case (n=1529)	2874	184	1348	178	3		
	control (n= 1439)	2763	111	1328	107	4	0.242	Chinese
Mohaddese et al.(2012)	case (n= 125)	187	63	62	63	0		
	control (n= 125)	177	73	53	71	1	0.00002	E. Azerbaijan
Zheng et al.(2012)	case (n= 227)	244	210	65	114	48		
	control (n= 152)	172	132	48	76	28	0.828	Chinese
Kommoju et al.(2013)	case (n= 758)	1168	318	440	288	30		
	control (n= 60)	1046	319	379	211	31	0.812	S. Indian
Bazzi et al.(2014)	case (n= 90)	145	33	62	21	6		
	control (n= 96)	164	28	69	26	1	0.393	Saudi
Fghih et al.(2014)	case (n=151)	231	71	90	51	10		
	control (n= (155)	227	83	89	49	17	0.015	Iranian

RESULTS

Included studies

The study selection process is shown as flow chart fig 1. After review, 14 studies (7-20) from 8 populations met inclusion criteria with 9232 T2DM patients and 8384 controls. This study was also sub-grouped as follows: E. Asian (7-9, 11, 12, 15, and 17), W. Asian (16, 19, and 20) and S. Asian (10, 13, and 18). Table 1summarized the characteristics of the study and distribution of SLC30A8 C/T (rs 13266634) genotypes and alleles among T2DM patients and controls.

Fig 2:Forest plots for association between SLC30A8 C/T gene polymorphism and T2DM:A) allele model (Avs T); B) dominat model (CC vs CT+ TT); C) additive model (CC + TT) and D) recessive model (CC + CT vs TT). The area of the squares reflects the study specific weight. The diamond shows the summary random-effects odds ratio estimate from 14 studies

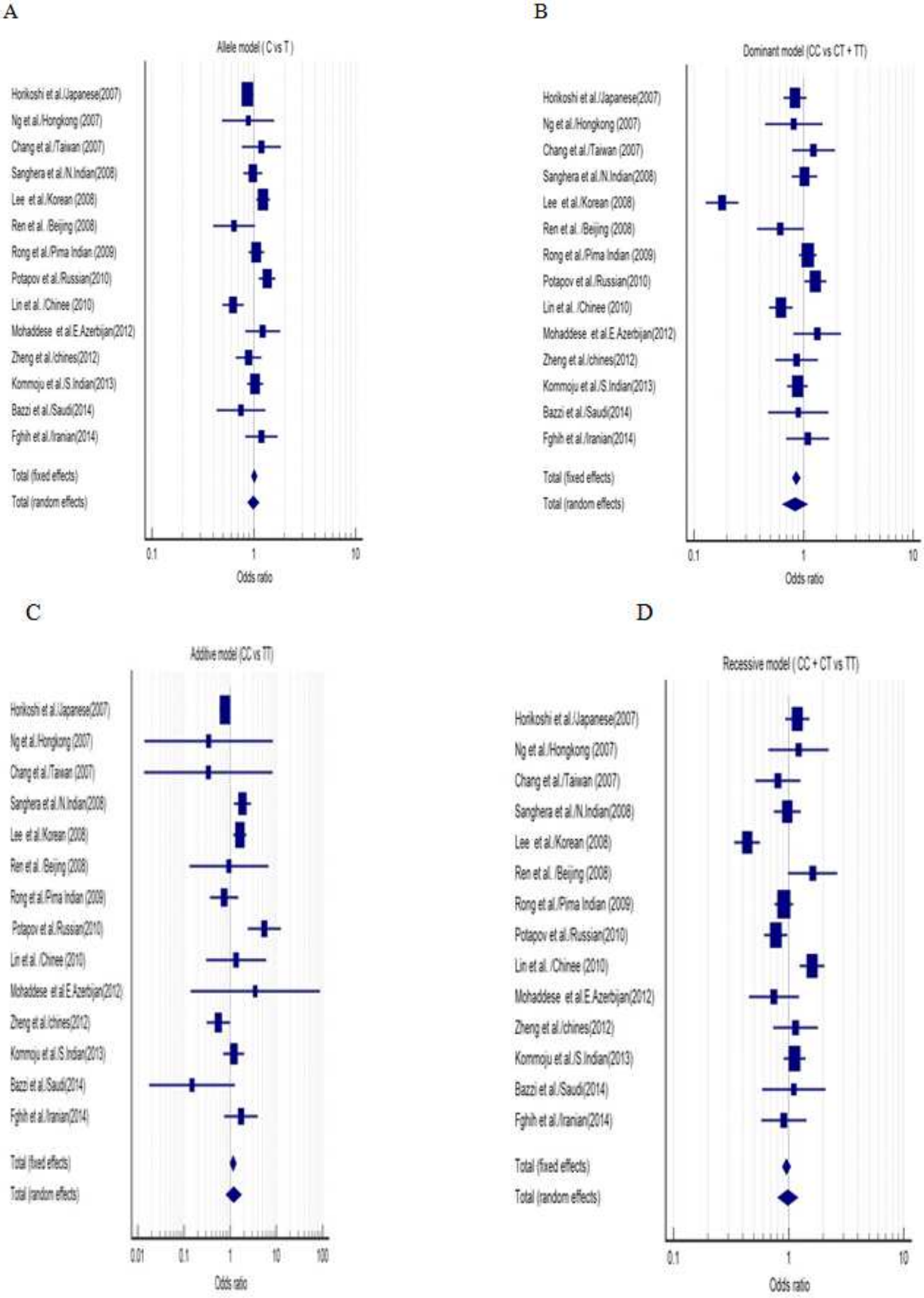


Table 2: Summary risk estimates for association between SLC30A8 rs13266634 C>T polymorphism and T2DM

Genetic contrasts	Ethnic group	Studies (n)	OR (95% CI)	P value	Heterogeneity		
					Q- test	I^2 (%)	P value
C vs T	Overall	14	0.951 (0.895 - 1.01)	P=0.101	117.87	88.97	P<0.0001
	W. Asian	3	1.1 (0.865 - 1.4)	P=0.437	2.3121	13.5	P=0.3147
	E. Asian	7	0.93 (0.854 - 1.013)	P=0.97	27.72	78.36	P<0.0001
	S. Asian	3	1.032 (0.926 - 1.151)	P=0.572	0.3262	0	P=0.8495
CC vs TT	Overall	14	1.16 (0.997 -1.35)	P=0.055	44.6	70.85	P<0.0001
	W. Asian	3	1.149(0.576 - 2.29)	P=0.693	4.82	58.54	P=0.0896
	E. Asian	7	0.967 (0.799 - 1.17)	P=0.731	16.934	64.57	P<0.0095
	S. Asian	3	1.35 (1.005 - 1.825)	**P<0.047**	4.89	59.1	P=0.0867
CC vs CT+TT	Overall	14	0.858 (0.792 - 0.929)	**P<0.001**	113.68	88.56	P<0.0001
	W. Asian	3	1.125 (0.836 - 1.514)	P=0.436	0.957	0	P=0.619
	E. Asian	7	0.612 (0.539 - 0.696)	**P<0.001**	66.91	91.03	P<0.0001
	S. Asian	3	1.006 (0.887 - 1.140)	P=0.929	2.24	10.66	P=0.3265
CC+CT vs TT	Overall	14	0.958 (0.886 - 1.037)	P=0.289	68.26	80.96	P<0.0001
	W. Asian	3	0.889(.661 - 1.196)	P=0.436	0.96	0	P=0.62
	E. Asian	7	0.995 (0.881 - 1.122)	P=0.931	60.6	90.1	P<0.0001
	S. Asian	3	0.994 (0.877 - 1.127)	P=0.929	2.24	10.66	P=0.3265

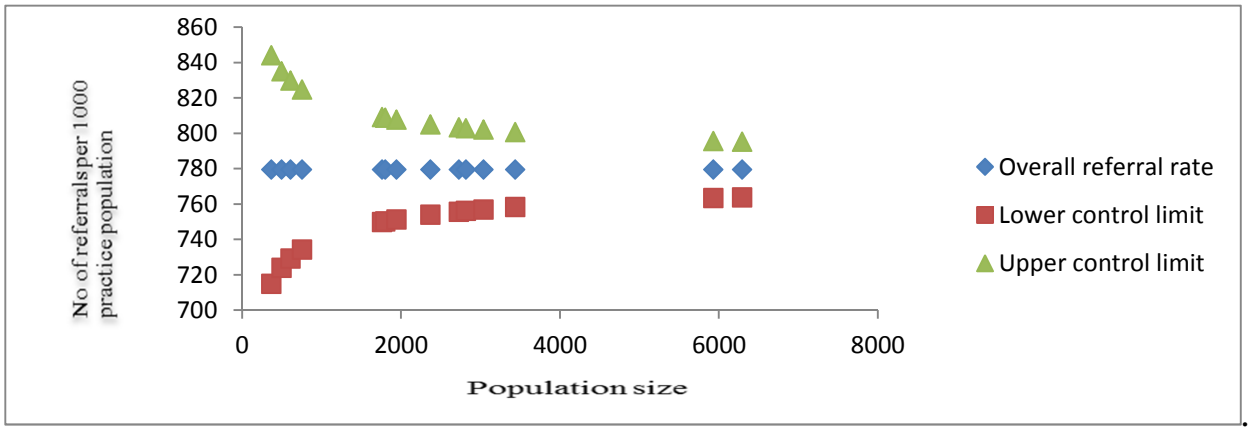
Association of SLC30A8 C/T (rs13266634) and T2DM risk

The combined overall analysis revealed that the CC genotype showed a significant association between SLC30A8 C/T polymorphism and T2DM (Dominant model: OR=0.858; 95% CI=0.792-0.929; P<0.001) Similarly the C allele (Allelic model: OR=0.958, 95% CI=0.886-1.037, P=0.289) and CT heterozygote (Recessive model: OR=0.951, 95% CI=0.895-1.010, P=0.101) also explained the risk of C allele with diabetes. In subgroup dominant model frequency comparison also, the results indicated that rs13266634 C/T polymorphism was significantly associated with increased T2DM risk in E. Asians (OR = 0.612, 95% CI: 0.539 –0.6996, P < 0.001). Similarly, the Additive model revealed association of C/T with T2DM risk in S. Asians (OR = 0.967, 95% CI: 0.799 –1.17, P < 0.047). And when the pooled odds ratio for rs13266634 C/T polymorphism and T2DM risk was calculated under a random-effects model, the results of overall Asians and Sub groups of the Asians (E. Asian, S. Asian, and W. Asian) were similar to those under a fixed-effects model (Table 1).

Heterogeneity test and publication bias

HWE test was performed to detect the genetic equilibrium of each study. The genotypic distributions in the controls of all included studies were in agreement with HWE except for the three studies, Japanese (1), Beijing (15) and E. Azerbaijan (19). In the sub-group population analysis there was heterogeneity observed between several groups. But interestingly, heterogeneity was not observed among the S. Asian populations (Table 2). Publication bias was calculated based on funnel plot representation (Fig: 3) and the shape of funnel plot seemed symmetrical showing the evidence of no bias.

Fig. 3 Funnel plot evaluation for publication bias in Asian T2DM patients and SLC30A8 rs13266634 C>T polymorphism studies



DISCUSSION

The pathogenesis of T2DM is complex and genetic factors play a key role in the disease susceptibility. Mutation of SLC30A8 is associated with dysfunction of pancreatic β-cells. Recently number of studies has reported the importance of ZnT8A in the development of diabetes. In most genetic studies, the main functional effect of this polymorphism was a decreased insulin release (21, 22). It was observed in animal model that the Arg325 form(C

allele) of ZnT8 is less active as Zinc transporter (23). The association between SLC30A8 gene and T2DM has been also confirmed in other meta-analysis (24). The present meta-analysis which was focused on the association between rs 13266634 C/T polymorphism and T2DM analyzing studies carried out in Asian population revealed that genetic variations are significant in T2DM. The data from published studies in this geographic region were combined to estimate genetic association using various models, among which the dominant model showed significance (OR=0.858; 95% CI=0.792-0.929; P<0.001). Further the sub-group analysis among Asian population according to ethnicity, showed significant association in dominant model (OR = 0.612, 95% CI: 0.539 –0.6996, P < 0.001) among E. Asians and Additive model (OR = 0.967, 95% CI: 0.799 –1.17, P < 0.047) among S. Asians. Recently a meta-analysis study by Tan et al. (2009) has also detected a heterogeneity between Asian populations (I^2=39%). The SLC30A8 C/T rs13266634 risk allele has an estimated prevalence of 55% in Asians, 75% in Europeans according to Hap-Map data attributing a risk of T2DM around 9.5% in Europeans and 8.1% in East Asians (25). In conclusion the SLC30A8 rs 13266634 polymorphism is among the most replicated genetic markers of T2DM and it has a specific role in Asian population (26). In the present meta-analysis, the C allele of SLC30A8 C/T polymorphism was associated with the risk of T2DM in Asian populations. However, larger prospective studies are needed to validate such meta-analysis.

Acknowledgement
The study was supported by UGC - NRCBS, CAS, DST-PURSE, and UGC – Meritorious fellowship. We are grateful to Miss. Sony Kadiam, Research Scholar, Dept. of Immunology, Madurai Kamaraj University, India for her valuable assistance for writing this manuscript.

REFERENCES

[1] M.A. Permutt; J. Wasson; N. Cox, *J Clin Invest*, **2005**, 115, 1431- 9.
[2] J.R. Perry; T.M. Frayling, *Curr Opin Clin Nutr Metab Care*, **2008**, 11, 371-7.
[3] F. Chimienti; S. Devergnas; A. Favier; M. Seve , *Diabetes*, **2004**,53, 2330–7.
[4] F.Chimienti; A. Favier; M. Seve, *Biometals*, **2005**,18, 313–7.
[5] N.D. Palmer; M.O. Goodarzi;C.D. Langefeld; J. Ziegler; J.M. Norris; D.M. Haffner, *Diabetes* **2008**, 57, 1093–100.
[6] V. Steinthorsdottir; G. Thorleifsson; I. Reynisdottir; R. Benediktsson; T. Jonsdottir; G.B. Walters, *Nat Genet* **2007**,39, 770–5.
[7] M. Horikoshi; K. Hara; C. Ito, N. Shojima; R. Nagai; K. Ueki, *Diabetologia*, **2007**, 50, 2461-6.
[8] YH. Lee; ES. Kang; SH. Kim; SJ. Han:CH. Kim; HJ Kim , *J Hum Genet*, **2008**, 53, 991-8.
[9] MC. Ng; KS. Park;B. Oh; CH. Tam; CYM. Cho; HD , *Diabetes* **2008**, 57, 2226-33.
[10] R. Rong;RL. Hanson; D. Ortiz; C. Wiedrich; S. Kobes; WC. Knowler, *Diabetes* **2009**, 5, 8478-88.
[11] Y. Lin; P. Li; L. Cai, *BMC Med Genet*, **2010**, 11, 97.
[12] X. Zheng; Ren W, S. Zhang; *Mol Biol Rep*. **2012**, 39(1),17-23.
[13] DK.Sanghera; Ortega L; Han S, Singh J; Ralhan SK; Wander GS, *BMC Med Genet*, **2008**, 9(59),1–9.
[14] V.A. Potapov; M.S.Chistiakov;M.V. Shamkhalova; V.V. Shestakova, *Diabetes India*, **2009**, 219–223.
[15] S.M. Mohaddes,F. Karami, J. Gharesouran, and A. Bahrami, *Journal of Sciences*, **2012**, 23(1), 15-20.
[16] H. Faghih; S. Khatami; N. Azarpira; A. Foroughmand, *Mol Biol Rep*,**2014**, 41, 2709–2715.
[17] M.D. Bazzi; F.A. Nasr; M.S. Alanazi; A. Alamri; A.A. Turjoman; A.S. Moustafa; A.A. Alfadda; A.A.K. Pathan and N.R. Parine, *Genet. Mol. Res*, **2014**, 13 (4), 10194-10203.
[18] U.J. Kommoju; J. Maruda; S. Kadarkarai; K. Irgam; J. P. Kotla; L. Velaga; B. M. Reddy, *Meta Gene*, **2013**, 1, 15–23.
[19] Q. Ren; XY. Han; F. Wang; XY. Zhang; LC. Han;YY. Luo; XH. Zhou; L.N. Ji, *Diabetologia*, **2008**, 51(7), 1146-1152.
[20] YC. Chang; TJ.Chang; YD. Jiang; S. Kuo; KC. Lee; KC.Chiu; LM. Chuang , *Diabetes* **2007**, 56(10),2631-2637.
[21] V. Lyssenko; A. Jonsson; P. Almgren; N. Pulizzi; B. Isomaa ; T. Tuomi T, *N Engl J Med* , **2008**, 359, 2220-32.
[22] H. Staiger; F. Machicao; N. Stefan; O. Tschritter; C. Thamer; K. Kantartzis, *PLoS One*, **2007**, 2. e832.
[23] TJ.Nicolson; EA. Bellomo; N. Wijesekara; MK. Loder; JM. Baldwin; AV. Gyulkhandanyan, *Diabetes*, **2009**, 58, 2070-83.
[24] S. Cauchi; S. Del Guerra; H. Choquet ; V. D'Aleo; CJ. Groves; R. Lupi, *Mol Genet Metab*, 2**0**10, 100(1), 77–82.
[25] YL. Jing; QM. Sun; Y. Bi; SM. Shen; DL. Zhu, *Nutr Metab Cardiovasc Dis*, **2010** , Epub ahead of print].
[26] J.T. Tan; D.P. Ng; S. Nurbaya; S. Ye; X.L. Lim; H. Leong; L.T. Seet; W.F. Siew; W.Kon; T.Y. Wong; S.M. Saw; T. Aung; K.S. Chia; J. Lee; S.K. Chew; M. Seielstad; E.S.Tai, *J. Clin. Endocrinol. Metab.* **2009**.