

jcrpe-2018-0195

Original Article

DOI: 10.4274/jcrpe.0195

SLC30A8 Gene rs13266634 C/T Polymorphism in Type 1 Diabetes Children of Tamil Nadu, India

Thirunavukkarasu R et al. SLC30A8 Gene Polymorphism in Type 1 Diabetes

Ramasamy Thirunavukkarasu¹, Arthur Joseph Asirvatham², Ayyappan Chitra³,
Mariakuttikan Jayalakshmi¹

¹Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

²Department of Diabetology, Govt. Rajaji Hospital, Madurai, India

³Institute of Child health and Research Centre, Govt. Rajaji Hospital, Madurai, India

Address for Correspondence: Mariakuttikan Jayalakshmi MD, Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai-625021, India

E-mail: jayalakshmim@genomicsmku.org; jayalakshmimk11@yahoo.com

ORCID ID: orcid.org/

Conflict of interest: None declared

Received: 28.07.2018

Accepted: 07.09.2018

What is already known on this topic?

SLC30A8 rs13266634 C/T polymorphism in T1D patients from four different populations was previously reported. This gene polymorphism is associated with T1D in German population, but not in Danish, Japanese and British populations.

What this study adds?

To the authors best of knowledge this is the first family based report addressing on SLC30A8 gene polymorphism in T1D patients among South Indian population. The present study and meta-analysis shows that the rs13266634 C/T polymorphism is not associated with T1D.

Abstract

Objective: Zinc transporter 8 (ZnT8) is a multi-trans membrane protein situated in the insulin secretory granule of the islets of β -cells and is identified as a novel

auto-antigen in Type 1 diabetes (T1D). The gene coding for ZnT8 (solute carrier family 30 member 8; SLC30A8) is located in Chromosome 8q24.11. In this work it is aimed to identify the association of SLC30A8 rs13266634 C/T gene polymorphism with T1D in chosen children of Tamil Nadu, India. **Methods:** The family based study is made on 121 T1D patients and 214 of their family members as control. The SLC30A8 gene rs13266634 C/T polymorphism is evaluated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results:** No significant differences was observed in allele (OR=0.92; CI=0.33 - 2.58; p=0.88) and genotype (CC: p=0.74; CT: p=0.82; TT: p=0.80) frequencies of rs13266634 C/T between T1D patients and controls. Transmission disequilibrium test (TDT) has identified over-transmission of mutant T allele from parents to affected children (T: U = 9:7) without statistical significance. Meta-analysis on the overall effects of rs13266634 C allele frequency was not different (p=0.10 and $P_{\text{heterogeneity}} = 0.99$) in T1D patients upon comparison with control. **Conclusion:** The present study along with the meta-analysis does not show any substantial association of the rs13266634 C/T polymorphism with T1D development.

Keywords:

Type 1 diabetes · auto-antigen · polymorphisms · zinc transporter 8 Autoantibody · meta-analysis

Introduction

T1D is a complex, multifactorial disease caused by the selective destruction of insulin-producing pancreatic β -cells (1, 2). The autoimmune destruction of pancreatic β -cells by pathogenic T cells predominately targets the auto-antigens (3). The islet cell auto-antigens identified in T1D are ZnT8, glutamic acid decarboxylase 65 (GAD65), tyrosine phosphatase-related molecules-2 (IA-2) and insulin (4). ZnT8 is a multi-trans membrane protein belonging to a family of zinc transporter having a role in the transport of zinc ion generated from the cytoplasm or in to the insulin vesicles and plays a major role in insulin maturation (5). During the process of insulin biosynthesis and secretion, frequent exocytosis of glucose stimulated insulin secretion (ICSG) increase the chance of ZnT8 exposed to the β -cell surface (6), which further cause more ZnT8 antigen to be exposed. Once ZnT8 is exposed, it can trigger or exacerbate the ZnT8A (ZnT8 autoantibodies) in genetically susceptible individuals (7). Previous studies have reported autoantibodies to ZnT8 is highly prevalent among T1D children with new onset, and it could be a marker for disease risk (8,9,10,11). The cation efflux transporter ZnT8 may influence the development of ZnT8 immunogenicity and the phenotypic features of T1D. The *SLC30A8* gene located in *Chromosome 8q24.11* encodes for ZnT8 auto-antigen (ZnT8) and is made of 369 amino acids (12, 13). Notably,

aa268-369 of the cytoplasmic domain of ZnT8, especially ZnT8-325R and ZnT8-325W, is the dominant epitope in T1D. A common non-synonymous single-nucleotide polymorphism (SNP) of *SLC30A8*rs13266634 (C/T polymorphism), encodes either arginine (R) by the C allele or tryptophan (W) by the T allele at aa325 of ZnT8 (14) suggests that rs13266634 SNP might be critical for humoral autoimmunity in T1D (11,15). Thus, the present study opines that *SLC30A8* gene polymorphism is involved in T1D development.

The objective of this study is to investigate the association between rs13266634 C/T gene polymorphism and T1D among the children of Tamil Nadu and to apply these results in a meta-analysis to reveal the association between the *SLC30A8* risk allele and T1D for comparison in different ethnic groups.

Materials and Methods

Subjects

The study subjects comprised 121 T1D patients from the Dept. of Diabetology, Govt. Rajaji Hospital (GRH), Madurai, Tamil Nadu, India, along with 214 first degree relatives of them (120 parents and 94 siblings) as control. All individuals were evaluated based on the clinical history and routine laboratory test. The patients met the revised criteria of the American Diabetes Association (ADA) for the screening of T1D (16). Genomic DNA was extracted from 5mL of peripheral blood sample by salting out method (17). This study was approved by the institutional ethics committees (Ref. No. 23339/E4/3/10; MKU/IRB/11/11) and consented in writing by the participants.

Genotype analysis

Subjects were genotyped for rs13266634 C/T polymorphism of *SLC30A8* gene by PCR-RFLP (18, 19). The region surrounding the polymorphism was amplified with following primers, Forward, 5'-GGACAGAAAGAGTTCCCATAGCG-3'; Reverse, 5'-ATAGCAGCATGTTTGAAGGTGGC-3'. PCR was performed at 95°C for 5 min, followed by 40 cycles at 94°C for 40s and 69°C for 45s. A final extension step was carried out at 72°C for 5 min. The PCR products were digested using enzyme Msp1 (Thermo Scientific, USA) incubated at 37°C for 4 h and visualized on 2 % agarose gel. In wild-type genotype (CC) of the fragments obtained was of 234 and 195bp. In heterozygote genotype (CT), three fragments were detected as 429, 234, and 195bp. Where else, only one fragment with 429bp was identified in homozygote genotype (TT).

Meta-analysis

An extensive literature search was done to examine the association between T1D and *SLC30A8* gene. The original data were collected from following electronic databases: PubMed, Elsevier, Science Direct, Web of Science and Google Scholar with key words Zinc transporter protein member 8, ZnT8, *SLC30A8* gene polymorphism, *SLC30A8* or *SLC30A8* variant, combined with autoimmunity,

autoimmune diabetes, type 1 diabetes mellitus. All searches were done independently by more than two research investigators. Following inclusion criteria were applied: 1. Studies should be in case-control manner, 2. all patients met the diagnostic criteria for T1D according to American Diabetes Association (ADA), 3. the studies were excluded, if there was no report on genotype frequency or insufficient data.

Statistical analysis

The obtained clinical data were subjected to student t-test and χ^2 test after segregating the data based on age, number and sex of the subjects. Odds ratio (OR) and their p-values were calculated by logistic regression, which was performed using *STATA 14v*. In addition, the transmission/disequilibrium test (TDT) was employed to detect preferential transmission from heterozygous parents to affected offspring (20). The TDT analysis was done by *Haploview 4.2v*. The level of significance was set at $p \leq 0.05$. Heterogeneity evaluation was performed by the Cochran's Q-test (21) and $p < 0.10$ was considered statistically significant. If not significant, odds ratio (OR) and 95% confident interval (CI) was arrived by fixed effect model (22), otherwise random effect model to be used (23). Heterogeneity of the data was quantified using the I^2 test (24). I^2 value of 25, 50 and 75 % were nominally considered low, moderate and high estimate, respectively. Funnel plot and Egger's linear regression test was used for the analysis of publication bias (25). Meta-analysis was performed with *Rev Man 5.0v*.

Results

The demographic details of the T1D subjects and controls are given in Table 1. There was no significant differences observed in allele (OR=0.92; CI=0.33 - 2.58; $p=0.88$) and genotype (CC: OR=0.92; CI=0.58 - 1.47; $p=0.74$; CT: OR=1.05; CI=0.64 - 1.71; $p=0.82$; TT: OR =1.13; CI =0.42 - 3.00; $p=0.80$) frequencies of rs13266634 C/T between T1D patients and controls, respectively (Table 2). Upon analysis of 30 parent-offspring trios (one affected child and two parents from their family), of the study cohort, TDT analysis identified over-transmission of mutant T allele of rs13266634 C/T polymorphism from parents to affected children (T: U = 9:7; MAF=0.194; $\chi^2=0.25$; p value=0.61) without statistical significance. Meta-analysis of the data via literature survey was able to retrieve 18 studies. In the above, nine were excluded after screening the abstracts, review and irrelevant subject matter. Three studies provided incomprehensive information. Two studies were not considered as they provided insufficient genotype frequencies. Rest of the four studies (26, 27, 14, and 28) associated with rs13266634 C/T polymorphism in SLC30A8 gene of T1D which met the required criteria was taken for the present meta-analysis. Along with the present study, totally five eligible studies were included with a total of 10,376 T1D patients and 10,027 control subjects in the meta-analysis.

Characteristics of the said studies and the distribution of rs13266634 C/T genotypes and alleles in T1D patients and controls are given in Table 3. Overall effects of rs13266634 C allele frequency in T1D patients (OR = 0.97; CI = 0.92-1.01; p=0.10) based on pooled analysis were not different from controls (Fig.1). There was no evidence of virtual asymmetry ($\chi^2 = 0.29$; $I^2 = 0\%$; $P_{\text{heterogeneity}} = 0.99$) which indicated that no publication bias crept in the meta-analysis (Fig.2). The area of squares, horizontal lines and diamond shows the weight of specific study, confidence intervals and the summary of fixed-effects odds ratio, respectively. The open circle represents various studies considered for this plot correlation, no evidence of publication bias was found.

Discussion

The ZnT8 is highly expressed in the pancreatic islet β -cells and recognized as one of the four major auto-antigens in T1D patients. Autoantibodies are observed to be generated against ZnT8 prior to the onset of disease. It is known that rs13266634 C/T SNP is responsible for autoimmune response to ZnT8 (12). The rs13266634 C/T plays a susceptible role in the presence of impaired autoimmunity mediated β -cell dysfunction which leads to T1D development (13). Works on the role of rs13266634 C/T polymorphism in T1D among global population are scanty. This work appears to be the first family based TDT analysis on rs13266634 SNP with its allele transmission from parents to offspring. As for TDT results, the present study documents over-transmission of mutant T allele of rs13266634 in T1D. In case control scenario, the present study indicates that there is a lack of association of rs13266634 C/T polymorphism to T1D. Few earlier studies also lent support to this contention in Danish, Japanese and British population (26, 14, 28). However, a German study indicates the occurrence of the frequencies of C allele and CC genotype of rs13266634 C/T polymorphism higher in the early on-set of T1D patients than controls (27). Recent study revealed that an adjacent locus of rs2466293 in SLC30A8 gene seems to predispose to the risk of T1D in non-European descent (29).

Until now, several publications have gone in to the correlation of rs13266634 C/T polymorphisms with T1D. However the results remain inconclusive. In order to bring out a resolve opinion on this differential contention a meta-analysis was adapted with expanding sample size expecting to explore the relationship of polymorphism at rs13266634 C/T of the SLC30A8 gene having susceptibility to T1D. However the comprehension of the meta-analysis indicated that the C allele conferred no risk in the development of T1D. Nevertheless one of the previous meta-analyse on T2D revealed that the rs13266634 C/T polymorphism is significantly associated with impaired glucose tolerance (30).

Study Limitations

The study is limited by a relatively small number of subjects. Varied studies from different ethnicities with large sample size are required to firmly confirm the role of rs13266634 C/T polymorphism in T1D.

Conclusion

This result demonstrates that the allele, genotype, genetic models and allele transmission of rs13266634 C/T polymorphism are not heavily associated with T1D in the children of chosen Tamil Nadu population. Similarly, the meta-analysis also indicates that the rs13266634 C/T polymorphism was not associated with T1D.

Acknowledgments

This work was supported by the grants of the Tamil Nadu State Council for Science and Technology, Govt. of Tamil Nadu, India.

Ethics

Ethics Committee Approval: Ethic board consent for the study was approved by the institutional ethics committees of Govt. Rajaji Hospital (Ref. No. 23339/E4/3/10) and Madurai Kamaraj University (MKU/IRB/11/11).

Informed Consent: All parents were informed about the purpose of the study, and a signed consent for study participation was obtained.

Conflict of interest: None declared

Authorship Contributions

Concept: Mariakuttikan Jayalakshmi, Design: Mariakuttikan Jayalakshmi, Ramasamy Thirunavukkarasu, Data Collection or Processing: Ramasamy Thirunavukkarasu, Asirvatham Arthur Joseph, Ayyappan Chitra, Analysis or Interpretation: Ramasamy Thirunavukkarasu, Mariakuttikan Jayalakshmi, Literature Research: Ramasamy Thirunavukkarasu, Writing: Ramasamy Thirunavukkarasu, Mariakuttikan Jayalakshmi.

Financial Disclosure: This study was supported by the grants of the Tamil Nadu State Council for Science and Technology (ref. No. TNSCST/S&T Proj/ MS/ VR/2011-2012) by Govt. of Tamil Nadu and UGC - NRCBS, CAS, DST-PURSE, and UGC – Meritorious fellowship by Govt. of India.

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Figure 1 Forest plot depicting the association of SLC30A8 rs13266634 C-allele in T1D.

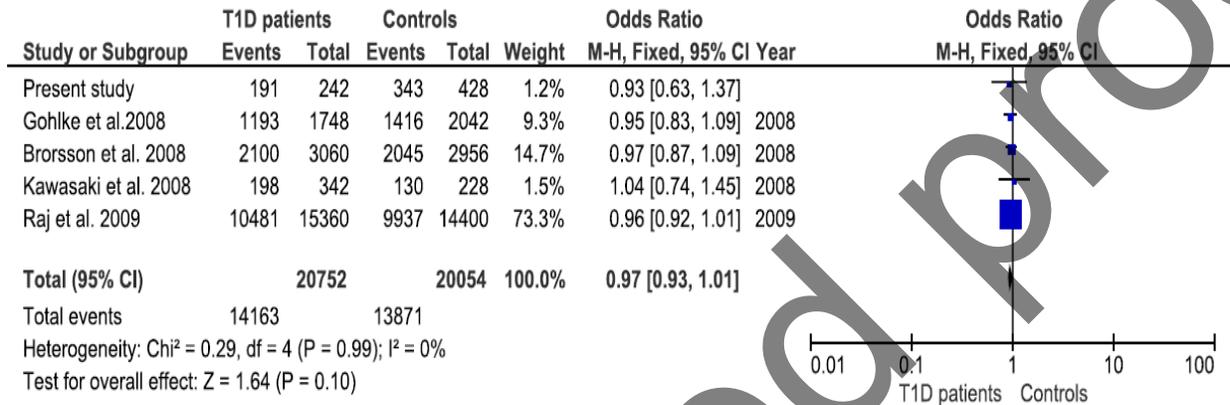


Figure 2. Begg’s funnel plot of SLC30A8 rs13266634 C/T with T1D patients in this meta-analysis

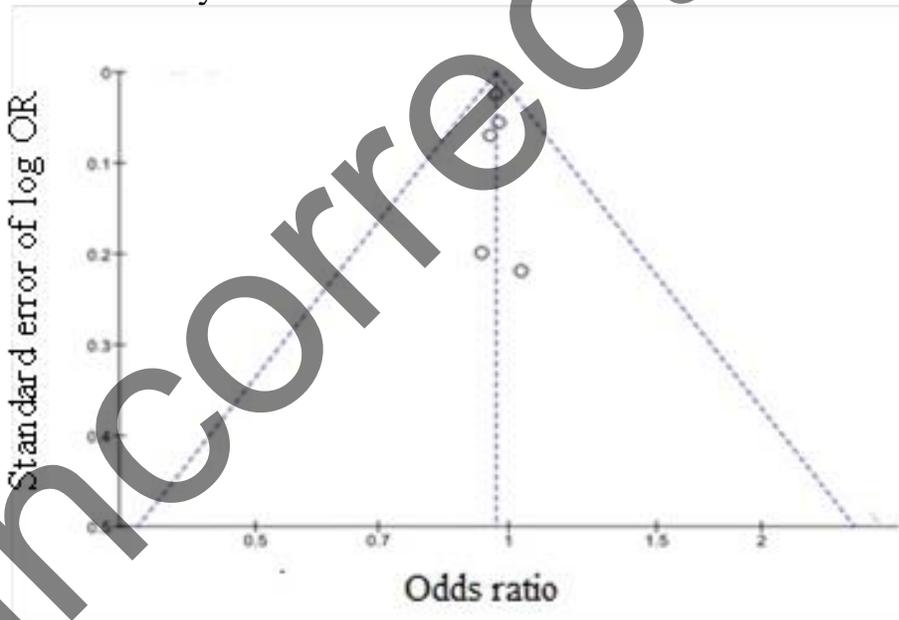


Table 1 Demographic details of the T1D patients and controls.

Details	T1D Patients		Controls
	Male	Female	

No. of Subjects (n)	70	51	214
Age (Year)	15.8±10.2	22.8±10.0	32.2±15.1
Age at diagnosis (Year)	15.5±8.4	14.8±7.9	-
TDDM (Year)	9.5±5.8	8.0±5.6	-

TDDM= Time duration of diabetes mellitus

Table 2 Comparison of SLC30A8 rs13266634C/T genotypes and allele frequencies between T1D patients and healthy controls

rs13266634C/T	Case (n=121)	Control (n=214)	OR (95% CI)	p-value
Genotype				
CC(RR)	77 (63%)	140 (65.4%)	0.92 (0.58-1.47)	0.74
CT (WW)	37 (30.5%)	63 (29.4%)	1.05 (0.64-1.71)	0.82
TT (WW)	7 (5.8%)	11 (5.2%)	1.13 (0.42-3.00)	0.80
CC+CT (RR+RW) ¹	114 (94.2%)	203 (94.9%)	0.88 (0.33-2.33)	0.80
CT+TT (RW+WW) ²	44 (36.4%)	74 (34.6%)	1.08 (0.67-1.72)	0.74
Allele				
C (R)	191 (78.9%)	343 (80.1%)	0.92 (0.33-2.58)	0.88
T (W)	51 (21.1%)	85 (19.9%)	-	-

OR = odds ratio; CI = confidence interval

confidence interval; n= number of sample

¹Dominant model (CC+CT vs TT)

²Recessive model (CT+TT vs CC)

Table 3 Distribution of SLC30A8 genotype and allele among T1D patients and controls, included for meta-analysis.

Study	Arms	C	T	CC	CT	TT	Population	Method
Present	Case (n-	191	51	77	37	7	South	PCR-RFLP

study	121)						Indian	
	Control (n-214)	343	85	14 0	63	11		
Gohlke et al. (2008)	Case (n-874)	1193	55 5	40 0	39 3	81	German	MOLDI-TOF-MS
	Control (n-1021)	1416	62 6	49 3	43 0	98		
Brorsson et al. (2008)	Case (n-1530)	2100	96 0	73 6	62 8	16 6	Danish	Sequencing & Taqman
	Control (n-1478)	2045	91 1	72 5	59 5	15 8		
Kawasaki et al. (2008)	Case (n-171)	198	14 4	63	72	36	Japanese	PCR-RFLP
	Control (n-114)	130	98	-	-	-		
Raj et al. (2009)	Case (n-7680)	1048 1	48 79	-	-	-	British	Taq-man PCR
	Control (n-7200)	9937	44 63	-	-	-		