Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 8 (3): 216-221
1st April, 2008

C-511T Polymorphism of Interleukin-1 β Gene is Not Associated in Type 2 Diabetes Mellitus-A Study in Malaysian Population

[1]R. Vasudevan, [1]Patimah Ismail,
[1]Johnson Stanslas and [2]Norashikin Shamsudin

The objective of this study is to determine the association between the C-511T polymorphism of Interleukin -1 β gene in T2DM with or without hypertension in Malaysian population. A total of 175 subjects were recruited for this study. Genotyping of C-511T variant was performed by Hot-start PCR-RFLP analysis. The frequency for CC, TC and TT genotypes of C-511T variant in IL-1 β gene was 25.45, 40 and 34.55% in T2DM, 25, 51.67 and 23.33% in T2DM with hypertension and 21.66, 36.67 and 41.67% respectively found in controls ($p>0.05$). This study suggests that there was no significant difference in the genotypic distribution of C-511T polymorphism of IL-1 β gene between T2DM and controls. Therefore, C-511T variant in IL-1 β gene polymorphism is not considered an independent risk factor or not a predictor for T2DM in Malaysian population.

Key words: Cytokines, interleukin -1 β gene, Type 2 Diabetes Mellitus, polymorphism, PCR-RFLP

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Patimah Ismail
Department of Biomedical Sciences,
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia,
Malaysia

Tel: 006-03-89472314
Fax: 00603 89436178

ANSInet
Asian Network for Scientific Information

[1]Molecular Biology Lab, Department of Biomedical Science,
[2]Department of Medicine, Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia, Malaysia

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a polygenic disorder has an impact on morbidity and mortality. T2DM is a growing concern in Malaysia (Ooyub *et al*., 2004). According to the Second National Health Morbidity Survey (1996), the prevalence of diabetes was 8.3% in Malaysia. The basis for this enlarged risk has been ascribed to a cluster of risk factors such as obesity; dyslipidemia and hypertension which often present in T2DM lead to coronary artery disease (United Kingdom Prospective Diabetes Study, 1998). Although environmental factors contribute to T2DM, the variants in amino acids of specific candidate genes were also susceptibility to T2DM (Anuradha *et al*., 2005; McCarthy, 2004). Besides those factors, an ethnic and geographical differences also varies in the prevalence of T2DM (Samanta *et al*., 1987).

Cytokines plays a pathological role in diabetes, particularly Interleukin-1 gene family of cytokine proteins (Tuttle *et al*., 2004; Mandrup-Poulsen *et al*., 1993). Proinflammatory cytokines such as Interleukin-1 alpha (IL-1 α) , Interleukin-1 beta (IL-1 β) and interleukin-1 receptor antagonist which belongs to Interleukin-1 family are invloved in both immune-mediated diseases and in the regulation of inflammation (Bidwell *et al*., 1999). A subclinical inflammation is associated with T2DM was due to dysregulation of the immune system (Joachim *et al*., 2003).

Genetic polymorphisms involved in IL-1 α and Interleukin-6 genes have been associated with various inflammatory diseases (Hollegaard and Bidwell, 2006). The polymorphism in IL-1 β gene has shown discrepancy results in different populations in association with type 1 diabetes (Kristiansen *et al*., 2000; Loughrey *et al*., 1998) but there is little information is available for IL-1 β in related to risk in T2DM. Interleukin-1 β gene has been mapped to Chr.2q13-q21. Several studies involved in C-to-T transition polymorphism at nucleotide position 511 in the promoter region of the IL-1 β gene in T2DM and in other diseases has been reported with controversial results in different populations (Achyut *et al*., 2007; Vohnout *et al*., 2003; Chou *et al*., 2005). To know the possible role of C-511T variant of IL-1 β gene towards susceptibility to T2DM in Malaysian population, the candidate gene approach method was employed in this study. To our knowledge, there have been no previous studies has been done in this polymorphism of Interleukin -1 β gene in T2DM in Malaysian Population. Therefore in this study, we determined the relationship of C-511T polymorphism of Interleukin -1 β gene in T2DM subjects in Malaysian population.

MATERIALS AND METHODS

Subjects: The study protocol was approved by the Ethical Committee of Faculty of Medical and Health Science; Universiti Putra Malaysia (UPM). Upon the approval, a total of 175 subjects were included under this study. Out of that, 115 patients were receiving treatment from April 2006-April 2007 were recruited from UPM Physician Clinic, Hospital Kuala Lumpur. The Subjects in this study were divided into three groups: T2DM (N-55) and T2DM with HPT (N-60) and controls (N-60). Type 2 Diabetes was defined as fasting plasma glucose >7.0 mmol L-1 and the levels were obtained from the medical records of all the subjects or in those currently receiving anti-diabetic therapy. Healthy individuals were collected randomly under the category of plasma glucose level was below 7.0 mmol L-1, resting Systolic Blood Pressure (SBP) <140 and Diastolic Blood Pressure (DBP) <90 mmHg on at least three separate occasions and had a negative family history diabetes and hypertension. Consent form was obtained from all the respondents, who involved in this study. Socio-Demographic and other risk factors were also assessed by using a questionnaire in both Malay and English language.

Biochemical analysis: Individual weight and height were obtained to calculate Body Mass Index (BMI) using the formula, weight (kg)/[height (m)]^2. Peripheral blood were drawn into an EDTA tube (Becton Dickinson, NJ) by a qualified phlebotomist. Separated plasma was stored at -20°C and then performed to determine the level of Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Total Cholesterol (TC) on Hitachi-912 Autoanalyser (Hitachi, Germany) using Roche Diagnostics kits (Mannheim, Germany). Low Density Lipoprotein Cholesterol (LDL-C) was calculated by Friedewald formula (Friedewald *et al*., 1972).

Genotyping methods: Genomic DNA was extracted from peripheral blood leukocytes using a genomic isolation DNA kit (BioBasic. Inc. Canada). The extracted DNA was quantified and the purification was checked using Biophotometer instrument (Eppendorf, Germany). The amplification of 304 bp of IL-1 β gene was done using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. PCR amplification was performed in a total volume of 25 μL reaction mixture contained 20 pmol of sense, 5'- TGG CAT TGA TCT GGTTCA TC - 3'; antisense, 5'- GTT TAG GAA TCT TCC CAC TT - 3' (synthesized by Research Biolabs, Malaysia) primer (Achyut *et al*., 2007) 0.4 mmol L-1 each dNTP, 2 mmol L-1, $MgCl_2$, 1x Taq buffer and 1 unit of NEB *Taq*

DNA polymerase (New England Biolabs, Beverly, MA, USA) and template DNA. PCR cycling conditions were performed for amplification under the following cycling conditions: initial denaturation step of 5 min at 94°C followed by 35 cycles of denaturation at 94°C (30 sec), annealing at 56°C (30 sec) and extension at 72°C (30 sec) and a final step of 5 min at 72°C (modified from Achyut *et al*., 2007) were carried out on iCycler instrument (BioRad Laboratories, Hercules, California, USA). The amplified PCR product was separated on agarose gel electrophoresis (Promega, Madison, USA). The PCR products was digested with 2 units of *AvaI* restriction enzyme (New England Biolabs, Beverly, MA, USA) at 37°C for 3 h with 1x NEB buffer 3, in a final volume of 20 μL reaction mixture. The PCR digested products were separated by electrophoresis on 4% agarose gel performed in Origins electrophoresis tank (Elchrom Scientific AG, Switzerland) and stained with ethidium bromide. The restricted fragments were visualized in Alpha Imager™ 1220 (Alpha Innotech, San Leandro, CA). The genotypes of C-511T polymorphism of IL-1 β gene show 304 bp for T/T; 304, 190 and 114 bp for C/T and for C/C genotypes was 190 and 114 bp. Approximately 5% of samples were genotyped at a separate occasions and shows identical results.

Statistical analysis: All the data of clinical characteristics of the subjects were expressed as mean±SD. Chi-Square test was performed to determine the distribution of genotypes and alleles was assessed for deviation from the Hardy-Weinberg equilibrium. Two-tailed student's t-test was performed for continuous variables to compare between the groups. All the statistical analysis was carried out by using SPSS (Chicago, IL) software version 13.0 for windows. A level of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Baseline characteristics: A total of 115 patients were recruited for this study, of whom 72 (62.61%) were males and 43 (37.39%) were females with mean age 57.98±10.06 year and healthy controls had 28 (46.67%) males/32 (53.33%) females: mean age 45.63±10.37 year. The clinical and biochemical characteristics of all the study subjects enrolled in this study have been shown in Table 1.

The majority of the subjects who participated in this study were males 100 (57.14%) compared to females 75 (42.86%). Among the entire subjects, Malay (n-70, 40.00%) race comprises more followed by Indians (n-60, 34.29%) and Chinese (n-45, 25.71%). Nearly 82 (68.33%) of the subjects had positive history of T2DM while controls were included on the absence of family history of T2DM. Significant differences ($p<0.001$) were observed in BMI 28.05±4.33 of T2DM subjects were compared to mean BMI 24.20±4.42 of controls. There was significant difference observed in diastolic blood pressure, glucose concentrations and HDL-C ($p<0.001$) however there was no significant difference found in other risk factors of T2DM such as LDL-C, total cholesterol and triglycerides ($p>0.05$).

C-511T polymorphism of interleukin-1β gene: In this study there was no statistically significant association observed between the C-511T variant of IL-1 β gene and T2DM subjects in Malaysian population. The PCR products of IL-1 β gene was digested with AvaI and the genotypes have been allotted on the basis of the pattern of bands on electrophoresis gel (Figure not shown). The distribution of genotypes of IL-1 β gene in Malaysian subjects showed no deviation from Hardy-Weinberg equilibrium ($p>0.05$). The genotypic and allelic distributions among the subjects are shown in Table 2.

In Malaysian population, the genotypic frequencies of IL-1 β gene for CC, CT, TT were 25.45, 40 and 34.55% respectively in T2DM (p-0.726), whereas in T2DM+HPT were 25, 51.67 and 23.33% respectively compared to 21.66, 36.67 and 41.67% for control subjects (p-0.092) respectively. The derived allele frequency for T allele were 54.55% in T2DM, 49.17% in T2DM+HPT as compared to 60.00% of control subjects respectively. It can be seen that the frequencies of IL-1 β gene was not associated with neither T2DM nor those with T2DM with Hypertension ($p>0.05$).

Table 1: Clinical characteristics of all the participants in this study

Parameters	Control (N-60)	T2DM (N-55)	p-value	T2DM with HPT (N-60)	p-value
Age	45.53±10.37	58.45±10.02	0.00	57.52±10.11	0.00
SBP	126.12±9.22	125.38±9.47	0.674	160.67±19.50	0.00
DBP	77.67±6.91	74.69±8.21	0.03	93.55±4.33	0.00
BMI	24.20±4.42	28.05±4.33	0.00	27.00±3.54	0.00
BGL	4.70±0.90	12.43±5.65	0.00	11.24±4.63	0.00
HDL	1.06±0.36	0.74±0.22	0.00	0.81±0.30	0.00
LDL	3.41±1.26	3.40±1.37	0.17	3.82±1.58	0.11
TG	1.72±1.01	2.05±1.04	0.95	1.99±1.03	0.16
TC	5.12±1.39	5.18±1.42	0.832	5.14±1.67	0.934

Significant t values obtained from two-tailed student's t-test. Results were expressed as Mean±SD BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; BGL: Blood Glucose Level; TC: Total Cholesterol; LDL: C-Low Density Lipoprotein Cholesterol; HDL: C- High Density Lipoprotein Cholesterol; TG: Triglycerides

Table 2: Genotype and Allele distributions between case and control subjects

Variables	Controls (N-60) n (%)	T2DM (N-55) n (%)	T2DM with HPT (N-60) n (%)
Genotypes			
CC	13 (21.66%)	14 (25.45%)	15 (25.0%)
CT	22(36.67%)	22 (40.0%)	31 (51.67%)
TT	25(41.67%)	19 (34.55%)	14 (23.33%)
Significance		0.726	0.092
Alleles			
C	48 (40.0%)	50 (45.45%)	61 (50.83%)
T	72 (60.0%)	60 (54.55%)	59 (49.17%)
Significance		0.426	0.120
Odds ratio (95% CI)	1.250 (0.740-2.110)	1.551 (0.930-2.586)	

Significance obtained through Chi-squared test by comparing cases with controls

Table 3: Genotypic and allelic distributions in male and female subjects

		Genotype				Alleles			
Group	Gender	CC	CT	TT	p-value	C	T	p-value	Odds ratio (95% CI)
T2DM (N-55)	M (N-32)	10	9	13	0.104*	29	35	0.972*	0.986 (0.461-2.111)
	F (N-23)	4	13	6		21	25		
T2DM with HPT (N-60)	M (N-40)	8	23	10	0.348*	39	43	0.078*	0.490 (0.221-1.089)
	F (N-20)	7	8	4		22	16		
Controls (N-60)	M (N-28)	7	8	13	0.474*	22	34	0.881*	0.946 (0.455-1.967)
	F (N-32)	6	14	12		26	38		

* No Significant (p>0.05), values were obtained through Chi-square test

Table 4: Genotype and allele distributions within the groups among the three ethnic races

		Genotype			
Race	n (%)	CC n (%)	CT n (%)	TT n (%)	p-value
T2DM (N-55)					
Malay	23 (41.82%)	8 (34.78%)	9 (39.13%)	6 (26.09%)	0.519
Chinese	7 (12.73%)	1 (14.29%)	4 (57.14%)	2 (28.57%)	
Indian	25 (45.45%)	5 (20.0%)	9 (36.0%)	11 (44.0%)	
T2DM with HPT (N-60)					
Malay	21 (35.0%)	7 (33.33%)	9 (42.86%)	5 (23.81%)	0.715
Chinese	13 (21.67%)	2 (15.38%)	7 (53.85%)	4 (30.77%)	
Indian	26 (43.33%)	6 (23.08%)	15 (57.69%)	5 (19.23%)	
Controls (N-60)					
Malay	26 (43.33%)	6 (23.08%)	7 (26.92%)	13 (50.0%)	0.601
Chinese	25 (41.67%)	6 (24.0%)	11 (44.0%)	8 (32.0%)	
Indian	9 (15%)	1 (11.12%)	4 (44.44%)	4 (44.44%)	

Significant value obtained through Chi-square test

The effect of gender on T2DM was also considered in this study for IL-1 β gene. Table 3, shows the genotype and allelic distributions of IL-1 β gene among males and females within the case and controls. There was no significant gender-mediated preferential distribution difference in either genotypic or allelic distribution in both male and female subjects (p>0.05) of IL-1 β gene in Malaysian population. The data was stratified according to the three main ethnic races in Malaysian population, we also found no significant difference (p>0.05) has been seen in the genotype frequencies between Malays, Chinese and Indians in either case or controls (Table 4).

To our knowledge, there have been no previous reports to determine the genotypes of - 511C/T variant of IL-1 β gene in Malaysian population. In this case-control study, we determined the association of IL-1 β gene in T2DM and in T2DM subjects with Hypertension in Malaysian population.

We found that-511C/T variant of IL-1 β gene was not associated with T2DM and in T2DM subjects with hypertension. In this study, the concentration of LDL-C of T2DM patients was not significantly differed from non-diabetic patients were well supported to previous studies (American Diabetes Association, 2002). In diabetic patients, the presence of increased TG level (>1.71 mmol L-1) and low HDL-Cholesterol (<1.04 mmol L-1) which greatly increases the risk of CVD supports the report of Laakso *et al.* (1993). Based on this study, gender, BMI and ethnicity were identified to be significantly important determinants of elevated LDL-Cholesterol and Triglycerides respectively, supports to previous studies (Ismail *et al.*, 2001; Mohamad *et al.*, 1997).

The variants involved in the pro-inflammatory cytokine reaction have been confirmed their association with Type 1 Diabetes (Loughrey *et al.*, 1998). However, the association in Type 2 Diabetes Mellitus in Malaysian population, revealed a negative association on -511C/T

polymorphism of IL-1 β gene. Indeed, we found no significant differences in the genotypes and alleles of -511C/T polymorphism within gender and races in case and control subjects. The findings of this research are in agreement with the previous studies in association with Type 1 Diabetes in Danish family (Kristiansen *et al*., 2000), coronary artery disease in Rome population (Vohnout *et al*., 2003) and rheumatic heart disease in Taiwan-Chinese population (Chou *et al*., 2005). In contrast, -511C/T variant of IL-1 β gene shows a possible role in T2DM among North Indian populations (Achyut *et al*., 2007). The variances are may be due to the ethnics or environmental factors which may contribute to the conflicting results (Persu, 2006). Present study has got some limitations. The sample size for this current study is only 200 subjects and relatively small as compare to genetic epidemiological studies. Moreover, the study subjects were heterogeneous population. Further study is needed with more sample size to know the prospective role of -511T allele of IL-1 β gene and other polymorphisms involved in pro-inflammatory cytokines in related to Type 2 Diabetes Mellitus was recommended in Malaysian population.

CONCLUSION

Our findings on -511C/T variant of IL-1 β gene does not provide adequate evidence on association with Type 2 Diabetes Mellitus and in T2DM subjects with Hypertension in Malaysian population. Therefore, -511T allele marker is not an independent risk on susceptibility to T2DM among Malaysian subjects.

ACKNOWLEDGMENTS

This study was supported by Ministry of Science, Technology and Environment (MOSTE), IRPA Project Grant No. 06-02-04-10054-EAR. The authors would like to extend their gratitude to all the volunteers who involved in this study.

REFERENCES

Achyut, B.R., A. Srivastava, S. Bhattacharya and B. Mittal, 2007. Genetic association of interleukin-1beta (-511C/T) and interleukin-1 receptor antagonist (86 bp repeat) polymorphisms with Type 2 diabetes mellitus in North Indians. Clin. Chim. Acta, 377 (1-2): 163-169.

American Diabetes Association, 2002. Management of dyslipidaemia in adults with diabetes (Position Statement). Diabetes Care, 25 (1): 74-77.

Anuradha, S., V. Radha, R. Deepa, T. Hansen, B. Carstensen and O. Pedersen, 2005. A prevalent amino acid polymorphism at codon 98 (Ala98Val) of the hepatocyte nuclear factor-1 alpha is associated with maturity-onset diabetes of the young and younger age at onset of type 2 diabetes in Asian Indians. Diabetes Care, 28 (10): 2430-2435.

Bidwell, J., L. Keen, G. Gallacher, R. Kimberly, T. Huizinga, M.F. McDermott, J. Oksenberg, J. McNicholl, F. Pociot, C. Hardt and S. D'Alfonso, 1999. Cytokine gene polymorphism in human disease: Online databases. Genes Immun., 1 (1): 3-19.

Chou, H.T., C.H. Tsai, W.C. Chen and F.J. Tsai, 2005. Lack of association of genetic polymorphisms in the interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4 and interleukin-10 genes with risk of rheumatic heart disease in Taiwan Chinese. Int. Heart J., 46 (3): 397-406.

Diabetes, National Health and Morbidity Survey, 1996. Institute of Public Health, Ministry of Health, 1997, Volume 9.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of low density lipoprotein cholesterol without the use of the preparative ultracentrifuge. Clin Chem., 18 (6): 499-502.

Hollegaard, M.V. and J.L. Bidwell, 2006. Cytokine gene polymorphism in human disease: On-line databases, Supplement 3. Genes Immun., 7 (4): 269-276.

Ismail, I.S., W. Nazaimoon, W. Muhamad, R. Letchuman, M. Singaraveloo, F.L. Hew, C. Shuguna and B.A.K. Khalid, 2001. Ethnicity and glycaemic control are major determinants of diabetic dyslipidaemia. Diabetic Med., 18 (6): 501-508.

Joachim, S., K. Anja and M. Matthias, 2003. Inflammatory cytokines and the risk to develop type 2 diabetes. Results of the prospective population-based european prospective investigation into cancer and nutrition (EPIC) - potsdam study. Diabetes, 52 (3): 812-817.

Kristiansen, O.P., F. Pociot, J. Johannesen, R. Bergholdt, C.A. Dinarello, L. Nerup and T. Mandrup-Poulsen, 2000. Linkage disequilibrium testing of four interleukin-1 gene-Cluster polymorphisms in Danish multiplex families with insulin-dependent diabetes mellitus. Cytokine, 12(2): 171-175.

Laakso, M., S. Lehto, I. Penttila and K. Pyorala, 1993. Lipids and lipoproteins predicting coronary heart disease mortality and morbidity in patients with non insulin-dependent diabetes. Circulation, 88 (4 Pt 1): 1421-1430.

Loughrey, B.V., A.P. Maxwell and D.G. Fogarty, 1998.. An interleukin 1β allele, which correlates with a high secretor phenotype is associated with diabetic nephropathy. Cytokine, 10 (12): 984-988.

Mandrup-Poulsen, T., U. Zumsteg, J. Reimers, F. Pociot, L. Morch, S. Helqvist, C.A. Dinarello and J. Nerup, 1993. Involvement of interleukin 1 and interleukin 1 antagonist in pancreatic beta-cell destruction in insulin-dependent diabetes mellitus. Cytokine, 5 (3): 185-191.

McCarthy, M.I., 2004. Progress in defining the molecular basis of type 2 diabetes through susceptibility gene identification. Hum. Mol. Genet., (Suppl. 1), 13 (1): 33-41.

Mohamad, M., F. Arshad, M.I. Mohd Noor and R. Ali, 1997. Prevalence of dyslipidaemia in non-insulin-dependent diabetic patients attending armed forces clinics in Kuala Lumpur. Asia Pacific J. Clin. Nutr. 6 (3): 203-206.

Ooyub, S., F. Ismail and N.A. Daud, 2004. Diabetes program in Malaysia - current and future, 2004. NCD Malaysia, 3 (2): 6-12.

Persu, A., 2006. Candidate gene studies: Accepting negative results. J. Hypertens, 24: 443-445.

Samanta, A., A.C. Burden and B. Fent, 1987. Comparative prevalence of insulin dependant diabetes mellitus in Asian and white Caucasian adults. Diabetes Res. Clin. Pract., 74 (1):1-6.

Tuttle, H.A., G. Davis-Gorman, S. Goldman, J.G. Copeland and P.F. McDonagh, 2004. Proinflammatory cytokines are increased in type 2 diabetic women with cardiovascular disease. J. Diabetes Complications, 18 (6): 343-351.

United Kingdom Prospective Diabetes Study (UKPDS), 1998. Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus (UKPDS 23). BMJ., 316 (7134): 823- 828.

Vohnout, B., A. Di Castelnuovo, R. Trotta, A. D'Orazi, G. Panniteri, A. Montali, M.B. Donati, M. Arca and L. Iacoviello, 2003. Interleukin-1 gene cluster polymorphisms and risk of coronary artery disease. Haematologica, 88 (1): 54-60.