ORIGINAL ARTICLE

Association of *TLL1* Gene Polymorphism (rs1503298, T > C) with Coronary Heart Disease in PREDICT, UDACS and ED Cohorts

Maryam Zain[1,2,5], Fazli Rabbi Awan[2,5], Jackie A. Cooper[1], Ka Wah Li[1], Jutta Palmen[1], Jay Acharya[1], Philip Howard[1], Shahid M. Baig[2,5], Robert S. Elkeles[3], Jeffrey W. Stephens[4], Helen Ireland[1] and Steve E. Humphries[1]

Abstract

Objective: To determine the sequence variant of *TLL1* gene (rs1503298, T > C) in three British cohorts (PREDICT, UDACS and ED) of patients with type-2 Diabetes mellitus (T2DM) in order to assess its association with coronary heart disease (CHD).

Study Design: Analytical study.

Place and Duration of Study: UCL, London, UK. Participants were genotyped in 2011-2012 for *TLL1* SNP. Samples and related information were previously collected in 2001-2003 for PREDICT, and in 2001-2002 for UDACS and ED groups.

Methodology: Patients included in PREDICT (n=600), UDACS (n=1020) and ED (n=1240) had Diabetes. *TLL1* SNP (rs1503298, T > C) was genotyped using TaqMan technology. Allele frequencies were compared using χ^2 test, and tested for Hardy-Weinberg equilibrium. The risk of disease was assessed from Odds ratios (OR) with 95% Confidence Intervals (95% CI). Moreover, for the PREDICT cohort, the SNP association was tested with Coronary Artery Calcification (CAC) scores.

Results: No significant association was found for this SNP with CHD or CAC scores in these cohorts.

Conclusion: This SNP could not be confirmed as a risk factor for CHD in T2DM patients. However, the low power of the small sample size available is a limitation to the modest effect on risk. Further studies in larger samples would be useful.

Key Words: *TLL1. rs1503298. Coronary heart disease. Diabetes mellitus. Coronary artery calcification (CAC) score.*

INTRODUCTION

Tolloid Like 1 (*TLL1*) gene is involved in heart development[1,2] and cellular processes relevant to both coronary heart disease (CHD) and Diabetes. It is one of the Peroxisome Proliferator Activator Receptor Gamma (PPARγ) activation pathway genes. PPARγ is the master regulator of lipid and glucose homeostasis, cardiac energy metabolism, vascular inflammation and cellular differentiation. PPARs have been implicated in the development of not only type-2 Diabetes mellitus (T2DM) but also in cardiovascular diseases.[3-7]

[1] *University College London, Faculty of Population Health Sciences, Institute of Cardiovascular Science, Centre for Cardiovascular Genetics, London, UK.*

[2] *Diabetes and Cardio-metabolic Disorders Laboratory, Human Molecular Genetics and Metabolic Disorders Group, Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan.*

[3] *Endocrinology and Metabolic Medicine, Imperial College London, St Mary's Hospital, London, UK.*

[4] *Diabetes Research Group, Institute of Life Sciences, College of Medicine, Swansea University, Swansea, UK.*

[5] *Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad, Pakistan.*

Correspondence: Dr. Fazli Rabbi Awan, Human Molecular Genetics and Metabolic Disorders Group, Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Jhang Road, P. O. Box. 577, Faisalabad. E-mail: awan.fr@gmail.com

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TLL1 gene is located on chromosome 4q32-33 and harbours rs1503298 SNP in the intron 12 of this gene. The T > C substitution in this SNP was studied with reference to CHD. A recent report involving 1045 patients with T2DM and testing the association of 3351 variants with CAD showed that in the White participants one SNP, rs1503298 in the *TLL1* gene, was significantly associated with the number of coronary lesions with percent diameter stenosis (DS) e20%. Patients with the homozygous minor CC genotype for the *TLL1* rs1503298 SNP had 22% more coronary lesions with at least 20% DS, as compared to those having TT genotype. Thus, CC genotype was associated with coronary heart complications and with an increase in coronary lesions in patients with T2DM having heart complications.[4]

Therefore, the objective of this study was to validate the already reported association of the gene variant (rs1503298 T > C) with CHD in Caucasian patients with T2DM and also to examine this association in other ethnic groups in the British population.

METHODOLOGY

The *TLL1* gene SNP rs1503298 (T > C) was genotyped in three British cohorts of T2DM patients. Ethical approval was obtained for these cohorts from respective authorities. The characteristics of these study cohorts are described below:

The Prospective Evaluation of Diabetic Ischemic heart disease by Computed Tomography study (PREDICT) is

a prospective study of patients (n=600) with established T2DM recruited from Diabetes clinics at several London hospitals, between November 2000 till November 2003. The participants were invited to the Royal Brompton Hospital, London for Electron Beam Computer Tomography (EBCT) to measure the Coronary Artery Calcification Score (CACS) at baseline in Hounsfield units (HU).[8] All the patients had Diabetes according to WHO criteria.[9] For those on insulin, T2DM was diagnosed on the basis of mode of presentation and history. The exclusion criteria were current and past history of CHD, uncontrolled hypertension, serious coexistent medical disorders likely to limit the life expectancy (e.g. cancer) or requiring extensive medical treatment and command of English insufficient to provide informed consent.

The University College Diabetes And Cardiovascular disease Study (UDACS) was a cross-sectional study designed to find the association between common variants in inflammatory / metabolic genes and biochemical risk factors implicated in CHD in patients with Diabetes. It was comprised of 1020 subjects consecutively recruited from the diabetes clinic at UCL Hospital in 2001-2002.[10] All patients had Diabetes according to WHO criteria and analysis was restricted to subjects with T2DM. The presence of CHD was recorded if any patient had positive coronary angiography/angioplasty, coronary artery bypass, cardiac thallium scan, exercise tolerance test, myocardial infarction, or symptomatic/treated angina. Any individual who was asymptomatic or had negative investigations was categorised as 'no CHD'.

The Ealing Diabetes study (ED) was a cross-sectional study comprising 1240 participants recruited consecutively from the diabetes clinic at Ealing Hospital in North West London in 2001-2002. Analysis was restricted to patients with T2DM.[11,12] Diagnostic criteria were the same for ED and UDACS.

For genotyping assays, the human genomic DNA was extracted by salting out method from blood nucleated cells as reported earlier.[13] The rs1503298 (T > C) SNP of *TLL1* gene was genotyped by TaqMan technology (Applied Bioscience, ABI, Warrington, UK). Reactions were performed on 384 well micro-plates and analysed using the ABI TaqMan 7900 HT software (Applied Bioscience).

Allele frequencies between the groups were compared using χ^2 test, and tested for the departure from Hardy-Weinberg equilibrium. The risk of CHD by genotype was assessed by calculating the Odds ratio (95% Confidence Interval) using logistic regression models. The baseline characteristics of the study participants were given as mean ± SD and compared by analysis of variance. Genetic associations were tested using an additive (per allele) model. CAC scores were presented as median with interquartile ranges and compared across genotypes using Kruskal-Wallis tests. Adjusted p-values were obtained using Tobit regression analysis which models scores of zero as a censored threshold (no detectable CAC) and assumes a normal distribution for the non-censored scores. In addition, CACS was expressed in categories approximating the presence of no, minimal, mild, moderate and severe coronary atherosclerosis (scores of 0, 1 - 10, 101 - 400, > 400 HU) and ordinal logistic regression was used to obtain the odds ratio for a higher CAC category according to genotype. All the statistical analysis were performed using STATA version 11 (StataCorp, TX, USA). The study is powered to detect an odds ratio for CHD of 1.85 in ED, 1.52 in UDACS and 1.4 for both studies combined with 80% power at the 5% significance level, based on an additive genetic model.

RESULTS

The ED study comprised of T2DM patients from different ethnic groups. Therefore, the genotype and C allele frequency in ethnic groups of this cohort was compared. Overall, there was a significant difference for genotype distribution in ethnic groups (p = 0.002). The genotype frequencies of rs1503298 (T > C) in various ethnic groups of this cohort were: Asians [TT = 112 (24.7%), TC = 229 (50.6%), CC = 112 (24.7%)], European Whites [TT = 96 (31.3%), TC = 152 (49.5%), CC = 59 (19.2%)], Africans [TT = 11 (12.9%), TC = 40 (47.1%), CC = 34 (40.0%)], and other ethnic groups which include Nigerians, Chinese [TT = 5 (27.8%), TC = 8 (44.4%), CC = 5 (27.8%)]. The C allele frequency (95% CI) was lower in Europeans {0.44 (0.40 - 0.48)}, compared to Asians {0.50 (0.46 - 0.53)} and Africans {0.63 (0.55 - 0.78), p = 0.002}.

The association of rs1503298 with CHD in the ED cohort was tested through a logistic regression analysis. Genotype and minor allele frequencies were examined within ethnic group for CHD positive and CHD negative groups. For each of the ethnic groups, per allele OR (95% CI) was calculated. No significant association of genotype with CHD was found for any of the ethnic groups in this cohort (Table I).

The UDACS group comprised of patients who had T2DM. The association of rs1503298 (T > C) with risk of cardiovascular disease was examined. As this group comprised of different ethnic groups (Blacks, Caucasians, Indians and some others), the genotype and allele frequencies were sorted in ethnic groups. The genotype frequencies of rs1503298 (T > C) in various ethnic groups of this cohort were: Blacks [TT = 4 (6.3%), TC = 33 (51.6%), CC = 27 (42.2%)], Caucasians [TT = 133 (27.3%), TC = 253 (52.0%), CC = 101 (20.7%)], Indians [TT = 23 (28.1%), TC = 35 (42.7%), CC = 24 (29.3%)], and few other ethnic groups [TT = 2 (16.7%), TC = 7 (58.3%), CC = 3 (25.0%)].

Table I: Association of CHD with *TLL1* SNP rs1503298 (T > C) in ED group.

Ethnic groups	*TLL1* SNP (rs1503298) Genotypes	CHD -ve N (%)	CHD +ve N (%)	[a]OR (95% CI) p-value
Asian	TT	78 (23.3)	34 (28.8)	0.91 (0.68 - 1.23)
	TC	175 (52.2)	54 (45.8)	p = 0.54
	CC	82 (24.5)	30 (25.4)	
	C allele freq. (95% CI)	0.50 (0.46 - 0.54)	0.48 (0.41 - 0.54)	
European whites (EW)	TT	81 (31.8)	15 (28.9)	1.01 (0.66 - 1.55)
	TC	124 (48.6)	28 (53.9)	p = 0.95
	CC	50 (19.6)	9 (17.3)	
	C allele freq. (95% CI)	0.43 (0.39 - 0.48)	0.44 (0.34 - 0.54)	
African (AFC)	TT	9 (12.7)	2 (14.3)	0.72 (0.29 - 1.83)
	TC	32 (45.1)	8 (57.1)	p = 0.57
	CC	30 (42.3)	4 (28.6)	
	C allele freq. (95% CI)	0.64 (0.56 - 0.72)	0.57 (0.37 - 0.75)	
All ethnic groups	TT	173 (25.6)	51 (27.4)	[b]0.95 (0.75 - 1.20)
	TC	339 (50.1)	90 (48.4)	p = 0.68
	CC	165 (24.4)	45 (24.2)	
	C allele freq.(95% CI)	0.54 (0.52 - 0.72)	0.46 (0.43 - 0.62)	

[a] Odds Ratio per C allele; [b] Odds Ratio per C allele adjusted for ethnic group.

Table II: Association of rs1503298 with CHD in UDACS cohort.

Ethnic groups	*TLL1* SNP (rs1503298) Genotypes	CHD -ve N (%)	CHD +ve N (%)	[a]OR (95% CI) p-value
Black	TT	4 (7.1)	0 (0)	[§]p = 0.81
	TC	30 (53.6)	3 (42.9)	
	CC	22 (39.3)	4 (57.1)	
	C allele freq. (95% CI)	0.66 (0.56 - 0.74)	0.78 (0.49 - 0.95)	
European whites / Caucasian	TT	90 (25.1)	41 (32.3)	[a]0.85 (0.63 - 1.14)
	TC	193 (53.9)	60 (47.2)	p = 0.29
	CC	75 (21.0)	26 (20.5)	
	C allele freq. (95% CI)	0.47 (0.44 - 0.51)	0.44 (0.37 - 0.50)	
Indians	TT	19 (31.2)	4 (19.1)	[a]1.09 (0.54 - 2.22)
	TC	23 (37.7)	12 (57.1)	p = 0.94
	CC	19 (31.2)	5 (23.8)	
	C allele freq. (95% CI)	0.50 (0.40 - 0.59)	0.52 (0.26 - 0.68)	
All ethnic groups	TT	115 (23.7)	45 (28.9)	[b]0.92 (0.70 - 1.19)
	TC	252 (51.9)	76 (48.7)	p = 0.51
	CC	119 (24.5)	35 (22.4)	
	C allele freq. (95% CI)	0.54 (0.47 - 0.72)	0.62 (0.43 - 0.72)	

[a] Odds Ratio (OR) per C allele, [b] OR per C allele adjusted for ethnic group. [§] This p-value was calculated from Fisher's exact test due to small numbers.

Table III: Distribution of rs1503298 (T > C) by CACS in PREDICT cohort.

	CACS in HU presented as N (%) in four categories					Odds ratio (95% CI)
TLL1 (rs1503298)	0	1 - 10	11 - 100	101 - 400	> 400	p-value
Caucasians						
TT	6 (26.1)	17 (26.6)	33 (35.5)	28 (27.7)	32 (30.8)	1.0 (0.77 - 1.30)[a]
TC	16 (69.6)	30 (46.9)	44 (47.3)	57 (56.4)	52 (50.0)	p = 0.996
CC	1 (4.4)	17 (26.6)	16 (17.2)	16 (15.8)	20 (19.2)	
S. Asians / Indians						
TT	5 (41.7)	4 (33.3)	4 (13.3)	7 (23.3)	8 (29.6)	0.93 (0.58 - 1.50)[a]
TC	5 (41.7)	5 (41.7)	15 (50.0)	15 (50.0)	14 (51.9)	p = 0.78
CC	2 (16.7)	2 (25.0)	11 (36.7)	8 (26.7)	5 (18.5)	
Others						
TT	1 (16.7)	2 (15.4)	5 (29.4)	1 (9.1)	1 (11.1)	0.94 (0.44 - 2.02)[a]
TC	4 (66.7)	6 (46.2)	11 (64.7)	8 (72.7)	6 (66.7)	p = 0.88
CC	1 (16.7)	5 (38.5)	1 (5.9)	2 (18.2)	2 (22.2)	
Combined ethnic group						
TT	12 (29.3)	23 (25.8)	42 (30.0)	36 (25.4)	41 (29.3)	1.00 (0.81 - 1.25)[b]
TC	25 (61.0)	41 (46.1)	70 (50.0)	80 (56.3)	72 (51.4)	p = 0.98
CC	4 (9.8)	25 (28.1)	28 (20.0)	26 (18.3)	27 (19.3)	

[a] Odds of a higher CACS category per C allele adjusted for age and gender, [b] Odds of a higher CACS category per C allele adjusted for age, gender, and ethnic group.

The C allele frequency was lower in Caucasians {0.46 (0.43 - 0.49)} compared to Blacks {0.68 (0.59 - 0.75)}, and Indians {0.50 (0.42 - 0.58)}. There was a significant (p = 0.001) difference in the genotype frequency by ethnic groups.

The association with CHD was also tested in UDACS. The allele and genotype frequencies for CHD positive and CHD negative groups as well as the OR (95% CI) for each of the ethnic groups and in all groups combined are given in Table II. There was no significant association of this SNP with CHD in any of the ethnic groups (Blacks, Caucasians, and Indians groups) individually as well as in combination. The odds ratios for UDACS and ED studies combined were 0.90 (0.70 - 1.15, p = 0.39) in Caucasians and 0.94 (0.79 - 1.12, p = 0.47) for all ethnic groups combined.

For PREDICT cohort, the mean age of the participants was 62.9 years. The group comprised of 68% males, 72% Caucasians, and 20% Asian Indians with duration of Diabetes 8 years or more. The allele and genotype frequencies for each of the ethnic group were tested as given below. The genotype frequencies of rs1503298 (T > C) in various ethnic groups of this cohort were: Caucasians [TT = 116 (30.1%), TC = 99 (51.7%), CC = 70 (18.2%)], Asians [TT = 28 (25.2%), TC = 54 (48.7%), CC = 29 (26.1%)], and other ethnic groups [TT = 10 (17.9%), TC = 35 (62.5%), CC = 11 (19.6%)]. The p value was not significant for the comparison of genotype frequency between each of the ethnic groups (p=0.13) C-allele frequencies were not significantly lower in Caucasians (0.44, 95% CI: 0.40 - 0.47), compared to Asians (0.50, 95% CI: 0.43 - 0.57) and other ethnic groups (0.50, 95% CI: 0.41 - 0.60).

The *TLL1* SNP (rs1503298) was tested for its association with CACS in the PREDICT study group. This group comprised of three different ethnic groups; Caucasians, Asians and other ethnic groups. P-values were not significant for any of the ethnic groups included in this cohort. The p-values obtained was p = 0.98 for Caucasians, 0.84 for Asians and 0.65 for all other ethnic groups.

Genotype distributions within the CACS categories in five ranges 0, 1 - 10, 11 - 100, 101 - 400 and greater than 400 HU are given in Table III. The Odds ratios for an increase in CACS category were not significant for any of the ethnic groups. The Odds ratio (OR, 95% CI, p-value) for Caucasians was 1.0 (95% CI 0.77 - 1.30, p = 0.996). Similarly, the other groups also gave non-significant results as: 0.93 (95% CI 0.58-1.90, p = 0.78) for South Asians / Indians, and 0.94 (95% CI 0.44 - 2.02, p = 0.88) for others. The OR (95% CI) for the combined ethnic groups result was 1.0 (95% CI 0.81 - 1.25, p = 0.98).

DISCUSSION

TLL1 SNP (rs1503298) has been previously reported to be associated with coronary heart disease (CHD). This sequence variant has been implicated in playing a role in cellular processes, which are related to CHD and T2DM. The *TLL1* gene is also involved in some other processes like PPAR gamma activation, which are regulators of lipids and glucose homeostasis, energy metabolism, vascular inflammation and cell differentiation.[4] The present study aimed at replicate such associations in three cohorts of patients with T2DM. For UDACS and ED cohorts, CHD status was examined at recruitment, and for the PREDICT study, extent of CAD was evaluated using CACS which has been shown to be a powerful predictor of cardiovascular events.

All three studies included different ethnic groups. The results were non-significant for all the groups individually as well as when tested in combination. Analysis of variance (ANOVA) was carried out to look at the biochemical parameters by genotype, but no significant results were found for any of the ethnic groups in the three cohorts (not shown). So most results do not agree with the previous study in which an increase in the extent of CAD with the rs1503298 variant was found.[4] Moreover, there are only few studies published to-date which show associations with some other diseases. In a study of chromosome 4q deletion syndrome for its association with congenital heart defects, *TLL1* gene has been identified using Illumina CytoSNP array platform as important player for congenital heart defects.[14] Another study of congenital heart defects in Chinese patients, *TLL1* mutation was observed in 6.1% of the patients as compared to controls.[15] Furthermore, in a recent study, SNPs in the *TLL1* gene have been associated with post-traumatic stress disorder.[16] In search for the genetic causes of congenital hyperinsulinism of infancy (CHI), a whole genome SNP genotyping and exome sequencing identified variants of *TLL1* gene in addition to a few others as the underlying cause.[17] Limited number of association studies for *TLL1* gene is probably due to the fact that this gene and its variants have been only recently explored for association studies. Hence, it is suggested that more studies in large and diverse population groups would be required to establish its precise association with cardiometabolic syndrome.

CONCLUSION

In UDACS and ED studies, no significant association with CHD was found. Also, in the PREDICT group no significant association with CAC score was observed in any ethnic group. However, although authors have not been able to confirm the association with CHD reported previously for this SNP, the low power of the small sample size available means, investigators of this study cannot rule out a modest effect on risk, and further studies in larger samples would be useful.

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REFERENCES

1. Stanczak P, Witecka J, Szydlo A, Gutmajster E, Lisik M, Augusciak-Duma A, *et al*. Mutations in mammalian tolloid-like 1 gene detected in adult patients with ASD. *Eur J Hum Genet* 2009; **17**:344-51.
2. Sabirzhanova I, Sabirzhanov B, Bjordahl J, Brandt J, Jay PY, Clark TG. Activation of Tolloid-like 1 gene expression by the cardiac specific homeobox gene Nkx2-5. *Dev Growth Differ* 2009; **51**:403-10.
3. Cresci S. The PPAR genes, cardiovascular disease and the emergence of PPAR pharmacogenetics. *Expert Opin Pharmacother* 2005; **6**:2577-91.
4. Cresci S, Wu J, Province MA, Spertus JA, Steffes M, McGill JB, *et al*. Peroxisome proliferator-activated receptor pathway gene polymorphism associated with extent of coronary artery disease in patients with type-2 diabetes in the bypass angioplasty revascularization investigation 2 diabetes trial. *Circulation* 2011; **124**:1426-34.
5. Libby P, Plutzky J. Inflammation in diabetes mellitus: role of peroxisome proliferator-activated receptor-alpha and peroxisome proliferator-activated receptor-gamma agonists. *Am J Cardiol* 2007; **99**:27B-40B.
6. Kim T, Yang Q. Peroxisome-proliferator-activated receptors regulate redox signaling in the cardiovascular system. *World J Cardiol* 2013; **5**:164-74.
7. Millar JS. Novel benefits of peroxisome proliferator-activated receptors on cardiovascular risk. *Curr Opin Lipidol* 2013; **24**: 233-8.
8. Elkeles RS, Feher MD, Flather MD, Godsland IF, Nugara F, Richmond W, *et al*. The association of coronary calcium score and conventional cardiovascular risk factors in type-2 diabetic subjects asymptomatic for coronary heart disease (The PREDICT Study). *Diabet Med* 2004; **21**:1129-34.
9. WHO. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1. diagnosis and classification of diabetes mellitus. Geneva: *World Health Organization;* 1999.
10. Dhamrait SS, Stephens JW, Cooper JA, Acharya J, Mani AR, Moore K, *et al*. Cardiovascular risk in healthy men and markers of oxidative stress in diabetic men are associated with common variation in the gene for uncoupling protein-2. *Eur Heart J* 2004; **25**:468-75.
11. Humphries SE, Gable D, Cooper JA, Ireland H, Stephens JW, Hurel SJ, *et al*. Common variants in the TCF7L-2 gene and predisposition to type-2 diabetes in UK European Whites, Indian Asians and Afro-Caribbean men and women. *J Mol Med (Berl)* 2006; **84**:1005-14.
12. Ireland H, Konstantoulas CJ, Cooper JA, Hawe E, Humphries SE, Mather H, *et al*. EPCR Ser219Gly: elevated sEPCR, prothrombin F1+2, risk for coronary heart disease, and increased sEPCR shedding *in vitro*. *Atherosclerosis* 2005; **183**: 283-92.
13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**:1215.
14. Xu W, Ahmad A, Dagenais S, Iyer RK, Innis JW. Chromosome 4q deletion syndrome: narrowing the cardiovascular critical region to 4q32.2-q34.3. *Am J Med Genet A* 2012; **158A**:635-40.
15. Li J, Ding JD, Fang X, Zhang H, Zheng RL, Cui JY, *et al*. Metalloproteinase Tolloid-like 1-gene mutation in Chinese patients with sporadic congenital heart diseases. *Zhonghua Xin Xue Guan Bing Za Zhi* 2012; **40**:402-5.
16. Xie P, Kranzler HR, Yang C, Zhao H, Farrer LA, Gelernter J. Genome-wide association study identifies new susceptibility loci for posttraumatic stress disorder. *Biol Psychiatry* 2013; **74**:656-63.
17. Proverbio MC, Mangano E, Gessi A, Bordoni R, Spinelli R, Asselta R, *et al*. Whole genome SNP genotyping and exome sequencing reveal novel genetic variants and putative causative genes in congenital hyperinsulinism. *PLoS One* 2013; **8**:e68740.