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ORIGINAL ARTICLE

Association of SRB1 and PON1 gene polymorphisms with type 2 diabetes mellitus: a case control study

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Abstract

Objective Single-nucleotide polymorphism (SNP) in Paraoxonase 1 (PON1) and scavenger receptor class b member 1 (SRB1) gene has been associated with impairing high-density lipoprotein (HDL) functionality as an antioxidant and shown to diminish ability of PON1 in cholesterol homeostasis. Several studies found that SRB1 and PON1 polymorphism increases T2DM risk. Our study aimed to investigate the association and susceptibility of polymorphic variants in SRB1 rs9919713 and PON1 rs662 with type 2 diabetes mellitus.

Methods In the present case-control study, 250 type 2 diabetes mellitus patients (T2DM) and 250 healthy volunteer were recruited. The genotypes of PON1 and SRB1 were determined by using polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) technique, and biochemical analysis was done using standard protocol.

Results C and R alleles showed significant association with T2DM susceptibility with an odds ratio of 1.42 ($p < 0.005$) and 1.40 ($p < 0.007$), respectively. The frequency of CC and RR genotype was significantly higher in T2DM patients compared with healthy controls. Furthermore, CC and RR genotypes were significantly associated with higher LDL and low HDL levels. Additionally, no other significant association was observed.

Conclusions We conclude that the PON1 and SRB1 gene polymorphisms may probably surrogate biomarkers for T2DM susceptibility.

Keywords Paraoxonase 1 · Polymorphism · Type 2 diabetes mellitus · Single-nucleotide polymorphism

Introduction

International Diabetes Federation (IDF) estimates that the number of diabetes patients in India had increased doubled from 19 million in 1995 to 40.9 million in 2007 [1] and projected to increase up to 69.9 million by 2025. Diabetes mellitus also known as non-insulin dependent diabetes mellitus is a complex metabolic disorder in which pancreatic beta cells become dysfunctional and cause insulin resistance or decreased production of insulin [2]. Previous genetic studies show that > 25 mutants are associated with type 2 diabetes mellitus, and preponderance is from coding and non-coding region which modulates insulin secretion [3, 4].

PON1 gene clustered on chromosome 7q21.3–22.1 is HDL associated enzyme composed of 354 amino acids with molecular weight of 43 KDa secreted primarily in the liver and non-steroidogenic tissues [5]. PON family is known to inhibit LDL oxidative modification and prevents the buildup of oxidized LDL by elevating cholesterol efflux [6]. It has been suggested that PON1 utilizes VLDL as a passageway to get into HDL. Low activity may confer increased risk of type 2 diabetes by impairing the ability of HDL to inhibit LDL oxidation, and gene variations found to decrease the ability of HDL to compel cholesterol efflux from macrophages and reverse cholesterol transport from peripheral tissues [7, 8]. Previous genetic studies show PON1 association with diabetes [9]. Polymorphism in R192Q shows the protective effect of HDL against LDL oxidation. 192QQ homozygous is most valuable in inhibiting the accumulation of lipid peroxides on LDL [6, 10].

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The SRB1 gene has been located on chromosome 12 covering a region of 75 kb comprising 13 exons. The gene encodes a receptor protein of nearly 80 kDa, whose weight can differ based on its amount of glycosylation [11]. SRB1 mainly functions as a receptor of HDL and loss of hepatic SRB1 leads to compositional changes in HDL including increased sphingomyelin, which markedly reduces the ability of LCAT to bind HDL leading to accumulation of toxic-free cholesterol (FC) in HDL, resulting in reduced cholesterol efflux capacity and reverse cholesterol transport RCT.

The present study was designed to investigate the association and susceptibility of polymorphic variants in SRB1 rs9919713 and PON1 rs662 with type 2 diabetes mellitus. These polymorphisms have been studied previously in different geographical region, but since the impact of polymorphisms on disease risk is known to differ from population to population, the effects of these polymorphisms on T2DM risk remained poorly understood among the Indians. There were a few previous studies on the association of PON1 polymorphisms with T2DM risk in the Indian population, but these studies had small sample sizes and thus were underpowered. The present work was also the first study to investigate the association of SRB1 rs9919713 polymorphism and T2DM susceptibility among Indians.

Material and methods

Subject selection

This population-based case control study included a total of 500 subjects recruited randomly from May 2015 to June 2016 with matched on age and ethnicity from out-patient Department of Medicine, King George's Medical University. Overall 250 T2DM subjects and 250 control subjects were recruited. Survey related to demographic details and family history of diabetes was attained from each subject. Body mass index (BMI) was calculated as the weight in kilogram divided by meter square of height. The entire group of patients recruited was informed regarding aim of the study, and written consent was obtained from each subject. Blood samples were taken from both the groups which were used for genotyping and biochemical analysis. A case for the present study was defined as a diagnosed case of type 2 diabetes mellitus with no medical history of cardiovascular or cerebrovascular diseases, cancer, and chronic renal, liver, heart disease. The inclusion criteria for the control subjects were the following; no history of diabetes mellitus and fasting plasma glucose is less than 110 mg/dL and HbA1c levels $\leq 5.8\%$.

Diagnostic criteria for type 2 diabetes

T2DM subjects were diagnosed on the basis of fasting blood sugar (FBS ≥ 126) and glycated hemoglobin (HbA1C $\geq 6.5\%$) level. Diagnosis and classification of diabetes was based on the guidelines of American Diabetes Association (ADA) [12].

Ethical clearance

Ethical consent was approved by institutional ethics committee's KGMU, Lucknow (Ref. Code: 71 ECM II B Thesis/P 13).

Biochemical examination

Biochemical parameters analysis of very low-density lipoprotein (VLDL) was determined by enzymatic method. Low-density lipoprotein (LDL) cholesterol levels were calculated by using the Friedewald formula [13]. Serum total cholesterol (TC), serum triglyceride (TG), and high-density lipoprotein (HDL) levels were assessed by XL-300 Transasia fully auto analyzer. HbA1c was measured using a semi auto analyzer (Transasia).

Blood sample collection

About 5 mL of venous blood was withdrawn under aseptic precautions after fasting for 10 h and distributed as follows: 2 mL of whole blood was put into EDTA vials (BD Vacutainer® spray-coated) mixed up and down gently and then used to measure the HbA1c and for isolation of genomic DNA. About 1 mL of whole blood was put into Na fluoride serum test tubes and centrifuged at 1500 rpm for 10 min. The separated serum was used for the assay of fasting blood sugar. About 2 mL of blood was placed in a plain tube without anticoagulant, and the tubes were left till coagulation. After coagulation, samples were centrifuged at 1500 rpm for 15 min. The separated serum was used for the assay of lipid profile.

DNA extraction

Genomic DNA from whole blood was isolated using a standardized phenol/chloroform extraction method [14].The quantity and quality of DNA were checked by UV spectrophotometry on a NanoDrop spectrophotometer and 0.8% (*w*/*v*) agarose gel electrophoresis, respectively.

Analysis of polymorphism

SRB1

The SRB1 rs9919713 polymorphism was analyzed by PCR followed by *RFLP technique*. Genomic DNA was amplified using the following PCR conditions: 95 °C for 5 min followed by 30 cycles of 95 °C for 40s, 64 °C for 38 s, 72 °C for 45 s, and final extension at 72 °C for 7 min with specific SRB1 gene forward primer 5'-CCTTGTTTTTCCTCGACGC-3′

and reverse primer 5-'CACCACCCCAGCCACCAGC-3′. Amplification was performed with 25 μL PCR reaction mixture containing 1.2 μL template DNA, 10 pmol of each primer, and 2X PCR master mixes (Thermo Scientific). The amplified 218 PCR product was digested with 1 U of restriction enzyme AgsI after incubation at 63 °C for 3 h and separated on 2% agarose gel in 1X TBE (Tris/Borate/EDTA) buffer by ethidium bromide staining and visualized under UV light by BIORAD gel doc system (Bio-Rad Laboratories, Inc.). As marker, 1 Kb DNA ladder was used. Thus, results demonstrate that in the case of SRB1 rs9919713 polymorphism, TT genotype is wild homozygote for the absence of the site (218 bp), CT genotype is heterozygote for the presence and absence of the site (218 and 187, 31 bp), and CC genotype is variant homozygote for the presence of the site (187 and 31 bp) Fig. 1.

PON1

The PON1 rs662 polymorphism was analyzed by following PCR conditions: 93 °C for 5 min followed by 30 cycles of 93 °*C* for 40s, 56 °*C* for 38 s, 71 °*C* for 45 s, and final extension at 73 °*C* for 7 min with specific PON1 forward primer 5'-AAACCCAAATACATCTCCCAGAAT-3′ and reverse primer 5'-GCTCCATCCCACATCTTGATTTTA-3′. Amplification was performed with 25 μL PCR reaction mixture containing 1.2 μL template DNA, 10 pmol of each primer, and 2X PCR master mixes (Thermo Scientific). The amplified PCR product was digested with 1 U of restriction enzyme Hinf1 after incubation at 39 °*C* for overnight and separated on 2% agarose gel in 1X TBE buffer by ethidium bromide staining and visualized under UV light by Bio-Rad gel doc system (Bio-Rad Laboratories, Inc.). The results demonstrate that in the case of PON1 rs662 polymorphism, QQ genotype is wild homozygote for the absence of the site (214 bp), QR genotype is heterozygote for the presence and absence of the site (214 and 190, 24 bp), and RR genotype is variant homozygote for the presence of the site (190 and 24 bp) Fig. 2.

Statistical analysis

Demographic and clinical data are reported as mean ± standard deviation (SD). Statistical comparisons between group means were analyzed using Mann-Whitney U test. Associations of genotypes with lipid parameters were analyzed by one-way ANOVA test. Association of PON1 and SRB1 genotypes with T2DM was done by odds ratio calculation. Allele and genotype frequencies were calculated by gene counting method. Observed genotype frequencies were compared with the expected frequency to confirm for Hardy-Weinberg equilibrium by Chi-square (χ^2) tests. $p \leq 0.05$ was considered significant. Statistical analysis was carried out using Statistical Program for Social Sciences (SPSS) version-19 (IBM SPSS Statistics, USA).

Results

Clinical and biochemical parameters as shown in Table 1 are compared to healthy control. No significant difference was observed in age, BMI between cases and controls. T2DM had significantly higher TC, TG, LDL, and VLDL, FBS,

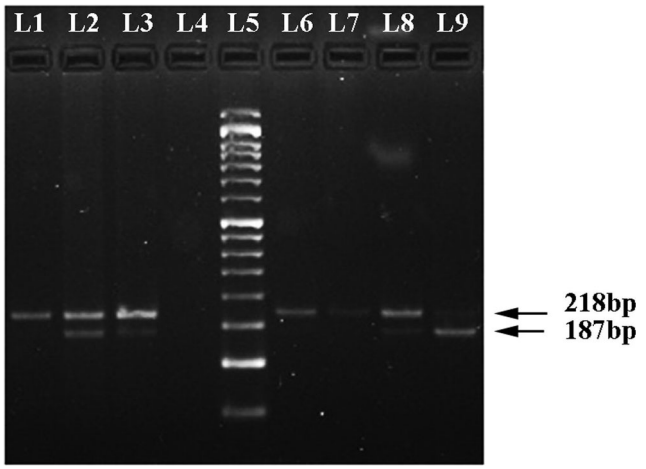

Fig. 1 Agarose gel electrophoresis for the rs9919713 polymorphism of scavenger receptor class B type 1 (SRB1) gene. The 218 bp bands correspond to wild homozygous TT genotype produced one fragment, while 187 bp and 218 bp corresponds to heterozygous CT that produced 3 fragments. The variant homozygous CC genotype produced 2 fragments of 187 bp and 31 bp. The 31 bp was invisible in the gel due to its fast migration speed. About 50 bp ladder marker (L1), TT genotype (L1, L6, L7), CT genotype (L2, L3, L8), and CC genotype (L9)

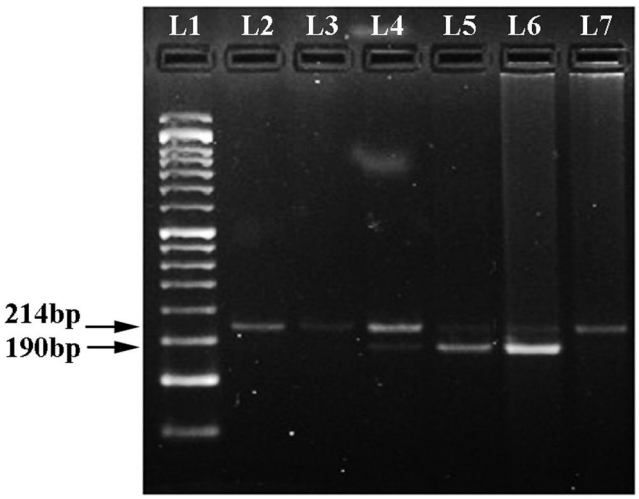

Fig. 2 Agarose gel electrophoresis for the rs662 polymorphism of paraoxonase 1 (PON1) gene. The 214 bp bands correspond to wild homozygous QQ genotype produced one fragment, while 190 bp, 214 bp, and 24 bp corresponds to heterozygous QR that produced 3 fragments. The 190 bp and 24 bp corresponds to variant homozygous RR genotype produced 2 fragments. The 24 bp was invisible in the gel due to its fast migration speed. About 50 bp ladder marker (L1), QQ genotype (L2, L3, L7), CT genotype (L4, L5), and RR genotype (L6)

Table 1 Clinical and biochemical parameters of controls and T2DM

Characteristics	Controls ($N = 250$)	T2DM ($N = 250$)	p value
Age (years)	47.5 ± 7.3	48.6 ± 9.7	0.11
BMI (kg/m^2)	24.8 ± 4.90	25.4 ± 5.10	0.14
HDL(mg/dl)	42.3 ± 8.5	36.5 ± 7.9	0.001*
LDL (mg/dl)	97.8 ± 30.7	139.2 ± 33.8	0.001*
VLDL(mg/dl)	30.8 ± 17.1	42.6 ± 10.6	0.001*
TG(mg/dl)	144.8 ± 79.4	206.1 ± 48.3	0.001*
FBS(mg/dl)	91.1 ± 11.7	149.1 ± 41.7	0.001*
HbA1c	5.6 ± 0.6	7.94 ± 0.99	0.001*

Data presented as mean ± SD; *Significant p value < 0.05

BMI body mass index, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *VLDL* very low-density lipoprotein, *TC* total cholesterol, *TG* triglycerides, *T2DM*, type 2 diabetes mellitus

Comparison between groups was performed with Mann-Whitney U test

and HbA1c. Furthermore, HDL levels were significantly reduced in T2DM patients when compared with controls.

Genotype and allele frequency of SRB1 and PON1

Both PON1 rs662 and SRB1 rs9919713 were consistent with Hardy-Weinberg equilibrium in all studied groups. In PON1 polymorphism, we observed R allele frequency was significantly associated with T2DM as compared to control with OR 1.40 ($\chi^2 = 7.09$, 95%CI = 1.09–1.80, $p = 0.007$). The frequency of RR genotype also showed significant increase in T2DM compared to controls with OR's 1.63 ($\chi^2 = 5.57$, 95% CI = 1.057–2.526, $p = 0.01$). R allele frequency was significantly increased in T2DM when compared to controls (46.4% vs. 38.6%, respectively), and it is associated with T2DM (Table 2). In SRB1 rs9919713 polymorphism, the frequency of CC ($\chi^2 = 7.47$, OR 2.0, 95%CI = 1.21–3.26, $p = 0.006$) genotypes was significantly increased in T2DM as compared to controls. The frequency of C allele was significantly associated with T2DM as compared to control with OR 1.42 ($\chi^2 = 7.74$, 95%CI = 1.11–1.82, $p = 0.005$) (Table 3).

Table 2 Comparison of genotypes and allele frequencies of PON1gene between healthy control and T2DM subjects

Genotype	Control $N = 250$	T2DM $N = 250$	OR (95% CI)	p value
QQ	118(47.2%)	93(37.2%)		
QR	75(30%)	81(32.4%)	1.37(0.904–2.076)	0.13 $\chi^2 = 2.21$
RR	57(22.8%)	76(30.4%)	1.63(1.057–2.526)	0.01* $\chi^2 = 5.57$
Allele				
Q	311(62.2%)	267(53.4%)		
R	193(38.6%)	233(46.4%)	1.40(1.09–1.80)	0.007* $\chi^2 = 7.09$

T2DM type 2 diabetes mellitus, *PON1* paraoxonase1

OR odds ratio, *CI* confidence interval; Comparison between group was performed with Chi-square test

*Significant p value < 0.05

Association of PON1 rs662 and SRB1 rs9919713 SNPs with lipid parameters

In PON1 polymorphism, QQ genotypes had higher HDL than the RR genotype. The levels of LDL were significantly increased in RR genotype as compared to QQ genotype (Table 4). Similarly, in SRB1 polymorphism, CC genotype had higher LDL level than TT genotypes. Further, HDL was significantly decreased in CC genotype (Table 5).

Discussion

The candidate gene method focuses on the association between genetic variations used in case control studies to find out the difference between allele and genotype frequency. SRB1 participates in the selective uptake of cholesterol ester [15] and binds a number of ligands with high affinity, including native HDL [16]. The selective uptake involves the transfer of cholesterol from the HDL particle and the release of the lipid-poor HDL particle into the plasma. In our previous findings, we observed significantly low level of HDL and PON1 protein level as the duration of diabetes increases. Numerous studies reported that PON1 and SRB1 play a significant role in worsening HDL's function and composition developing increased risk of T2DM.

Table 3 Comparison of genotypes and allele frequencies of SRB1 gene between healthy control and T2DM subjects

Genotype	Control $N = 250$	T2DM $N = 250$	OR (95% CI)	p value
TT	73(29.4%)	53(21%)	–	
CT	123(49%)	119(48%)	1.33(0.86–2.05)	0.19 $\chi^2 = 1.6$
CC	54(21.6%)	78(31%)	2 (1.21–3.26)	0.006* $\chi^2 = 7.47$
Allele				
T	269(53.8%)	225(45%)		
C	231(46.2%)	275(55%)	1.42 (1.11–1.82)	0.005* $\chi^2 = 7.74$

T2DM type 2 diabetes mellitus, *SRB1* scavenger receptor class B member I

OR odds ratio, *CI* confidence interval; Comparison between group was performed with Chi-square test

*Significant p value < 0.05

Table 4 Association of PON1 rs662 polymorphism with lipid parameters

Lipid parameter	QQ	QR	RR
Control	$N = 118$	$N = 75$	$N = 57$
LDL	79 ± 17.7	81.3 ± 17*	85.3 ± 18*
VLDL	34.5 ± 13.1	35.3 ± 13.2	33.2 ± 17.5
TC	137.4 ± 34.4	131.6 ± 33.4	135.3 ± 18.2
TG	179.1 ± 39.1	181.4 ± 33.5	184.1 ± 32
HDL	32.2 ± 11.7	34.2 ± 5.3	30.1 ± 7.5*
Cases	$N = 93$	$N = 81$	$N = 76$
LDL	81.3 ± 17	81.3 ± 17*	85.3 ± 18*
VLDL	43.4 ± 10.6	41.3 ± 10	43.7 ± 11.3
TC	178 ± 77.2	166.1 ± 67	176.5 ± 79.4
TG	215 ± 32.5	216.4 ± 31.5	220.4 ± 25.8
HDL	43.2 ± 9.7	41.1 ± 10.2	40.2 ± 10.8*

Data presented as mean ± SD; Genotypes were compared by one- way ANOVA

HDL high density lipoprotein, *LDL* low-density lipoprotein, *VLDL* very low-density lipoprotein, *TC* total cholesterol, *TG* triglycerides, *PON*1 paraoxonase 1

*Significant P value < 0.05

Present study examined the association of R allele with type 2 diabetes mellitus. The association of PON1 variant and the risk of type 2 diabetes mellitus have been confirmed in several previous studies [17, 18]. On the other side, some contradictory results did not find association with PON1 polymorphism and T2DM [19, 20]. Several ethnic studies informed that significant association was observed with T2DM in Saudi and Egyptian individuals [21, 22]. However, in south Iranian population, no significant correlation was observed [23]. These outcomes might be the result of different racial, biological region, lifestyle, and socioeconomic factors. We also believe that the inconsistency between our study and these erstwhile studies was due to the sample size employed.

Table 5 Association of SRB1 rs9919713 polymorphism with lipid parameters

Lipid parameters Control	TT $N = 73$	CT $N = 123$	CC $N = 54$
LDL	75.4 ± 20	77.3 ± 19.1	82.5 ± 17.1*
VLDL	41 ± 9.4	39.5 ± 9.1	43.1 ± 10.6
TC	172.1 ± 39.7	177 ± 29.6	176.8 ± 33.8
TG	144.2 ± 42	142.6 ± 75	147.5 ± 26.1
HDL	42.8 ± 11.5	39.5 ± 9.7*	39.7 ± 10.2*
Cases	$N = 53$	$N = 119$	$N = 78$
LDL	62.1 ± 16.2	64.2 ± 16.6	71.5 ± 15.5*
VLDL	41.4 ± 10	43.5 ± 10.2	44 ± 12.5
TC	222 ± 27.9	228.3 ± 21.1	216 ± 26.1
TG	179.7 ± 73.9	182.5 ± 79.2	176.3 ± 82.3
HDL	35.1 ± 7.5	33.1 ± 12.5	30.3 ± 7.2*

Data presented as mean ± SD; Genotypes were compared by one- way ANOVA

HDL high-density lipoprotein, *LDL* low-density lipoprotein, *VLDL* very low-density lipoprotein, *TC* total cholesterol, *TG* triglycerides, *SRB1* scavenger receptor class B member I

*Significant P value < 0.05

Additionally, Mackness et al. [20] reported that serum PON1 level was significantly lower in type 2 diabetes mellitus patients as compared to healthy individual, which is in steadiness with our earlier findings published by us [24]. The low levels of PON1 in T2DM might affect HDL functionality and decrease its ability to prevent LDL oxidation [19]. This implies that PON1 may reduce HDL activity in these individuals and also increase their susceptibility to T2DM. Recent studies demonstrated PON1 gene polymorphism and lipid parameters have also yielded conflicting results. Our findings also revealed that subjects carrying RR genotype had significantly higher levels of LDL. The results were in agreement with the outcomes of studies performed earlier [14]. But that is in contrast with the results of study that did not find association [19].

Moreover, In SRB1 gene polymorphism, we observed significant correlation of C allele with the risk of T2DM. Previously, findings proved that CC genotype had significant association with T2DM. We also observed lower frequencies of C allele in controls as compared to T2DM [23], which is in consistent with earlier findings that showed SRB1 rs9919713 gene polymorphism is associated with insulin resistance and T2DM [25]. Polymorphism may be implicated in the pathogenesis of insulin resistance by diminishing the concentration of PON1 and thus modulating the expression of GLUT-4. Earlier, human genetic studies confirmed that SRB1 carrying loss of function variant exhibit impaired cholesterol efflux to HDL. SRB1 polymorphism does not lead to change in amino acid sequence and cannot be linked at structure level. It is possible that some other variation in its region might effect, which is in linkage disequilibrium with SRB1 polymorphism and accountable for the observed association with T2DM.

Our results showed that increased HDL levels were associated with TT genotype in both the groups. We also found subjects carrying CC genotype had significantly increased LDL level. Our study supports previous findings that reported low HDL and high LDL levels in CC genotype [26, 27]. In a similar study of Tunisian population [28], T allele was found associated with higher HDL levels. However, some contradictory findings were reported in Chinese population [29]. Notwithstanding the clear functional evidence for an influence of PON1, SRB1 on HDL levels, the genetic epidemiological data is somewhat weak. Previous literature search identified polymorphic sites studied with HDL levels with varying results [30]. Large genetic epidemiological studies on this gene are required before a final conclusion can be drawn.

Conclusions

In conclusion, data suggested that the genetic variation in PON1 and SRB1 gene was independent influencing factors of diabetes mellitus and might be one of the candidate genes for conferring susceptibility to diabetes. The present study has certain limitations because it was conducted only on north Indian population with small sample size. Furthermore, we suggest study will be performed in larger sample size, in different ethnic groups in order to understand possible association of these loci alone or in linkage disequilibrium (LD) in development of diabetes which could possibly generate a more reliable result. However, efficient lifestyle modifications including implementation of a healthy dietary pattern like the Mediterranean diet, with physical activity, are vital in the prevention of type 2 diabetes. So, importance must be given to supporting a better lifestyle and finding solutions in order to increase devotion and compliance to the lifestyle modifications, especially for high-risk individuals.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

Informed consent The study was approved by King George's Medical University Ethics Committee and informed written consent was taken from all the subjects.

References

1. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australas Med J. 2014;7(1):45–8.
2. Ginsberg HN. Diabetic dyslipidemia: basic mechanisms underlying the common hypertriglyceridemia and low HDL cholesterol levels. Diabetes. 1996;45(3 SUPPL):27–30.
3. Cerda Á, et al. Influence of SCARB1 polymorphisms on serum lipids of hypercholesterolemic individuals treated with atorvastatin. Clin Chim Acta. 2010;411(9–10):631–7.
4. Prasad RB, Groop L. Genetics of type 2 diabetes—pitfalls and possibilities. Genes (Basel). 2015;6(1):87–123.
5. Gomathi P, Iyer AC, Murugan PS, Sasikumar S, Raj NBAJ, Ganesan D, et al. Association of paraoxonase-1 gene polymorphisms with insulin resistance in south Indian population. Gene. 2018;650:55–9.
6. Flekac M, Skrha J, Zídková K, Lacinová Z, Hilgertová J. Paraoxonase 1 gene polymorphisms and enzyme activities in diabetes mellitus. Physiol Res. 2008;57(5):717–26.
7. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress—a concise review. Saudi Pharm J. 2016;24(5):547–53.
8. Jayashree S, Arindam M, Vijay KV. Genetic epidemiology of coronary artery disease: an Asian Indian perspective. J Genet. 2015;94(3):539–49.
9. Macharia M, Kengne AP, Blackhurst DM, Erasmus RT, Matsha TE. Paraoxonase1 genetic polymorphisms in a mixed ancestry African population. Mediat Inflamm. 2014;2014.
10. Gokcen S. Serum paraoxonase levels and PON1(192) polymorphism in type 2 diabetes mellitus patients. Gazi Med J. 2013;24(3):70–3.
11. McCarthy JJ, et al. Polymorphisms of the scavenger receptor class B member 1 are associated with insulin resistance with evidence of gene by sex interaction. J Clin Endocrinol Metab. 2009;94(5):1789–96.
12. Care D, Suppl SS. 2. Classification and diagnosis of diabetes: standards of medical care in diabetesd2019. Diabetes Care. 2019;42(january):S13–28.
13. Kannan S, Mahadevan S, Ramji B, Jayapaul M, Kumaravel V. LDL-cholesterol: Friedewald calculated versus direct measurement-study from a large Indian laboratory database. Indian J Endocrinol Metab. 2014;18(4):502.
14. Gupta N, Binukumar BK, Singh S, Sunkaria A, Kandimalla R, Bhansali A, et al. Serum paraoxonase-1 (PON1) activities (PONase/AREase) and polymorphisms in patients with type 2 diabetes mellitus in a north-west Indian population. Gene. 2011;487(1):88–95.
15. Talmud PJ, Hawe E, Robertson K, Miller GJ, Miller NE. Genetic and environmental determinants of plasma high density lipoprotein cholesterol and apolipoprotein AI concentrations in healthy middle-aged men. Ann Hum Genet. 2002;66(2):111–124.
16. Yin RX, Wu DF, Aung LH, Yan TT, Cao XL, Long XJ, et al. Several lipid-related gene polymorphisms interact with overweight/obesity to modulate blood pressure levels. Int J Mol Sci. 2012;13(9):12062–81.
17. Bhaskar S, Ganesan M, Chandak GR, Mani R, Idris MM, Khaja N, et al. Association of *PON* 1 and *APOA* 5 gene polymorphisms in a cohort of Indian patients having coronary artery disease with and without type 2 diabetes. Genet Test Mol Biomarkers. 2011;15(7–8):507–12.
18. Shakeri R, Khajeniazi S, Marjani A. Association between promoter polymorphism (− 108C > T) of paraoxonase1 gene and it's paraoxonase activity in patients with Type2 diabetes in northern Iran. Clin Chim Acta. 2017;474(August):34–7.
19. Agachan B, Yilmaz H, Ergen HA, Karaali ZE, Isbir T. Paraoxonase (PON1) 55 and 192 polymorphism and its effects to oxidant-antioxidant system in Turkish patients with type 2 diabetes mellitus. Physiol Res. 2005;54(3):287–93.
20. Mackness B, Durrington PN, Abuashia B, Boulton AJ, Mackness MI. Low paraoxonase activity in type II diabetes mellitus complicated by retinopathy. Clin Sci (Lond). 2000;98:355–63.
21. Alharbi KK, Khan IA, al-Daghri NM, Munshi A, Sharma V, Mohammed AK, et al. ABCA1 C69T gene polymorphism and risk of type 2 diabetes mellitus in a Saudi population. J Biosci. 2013;38(5):893–7.
22. El-Lebedy D, Kafoury M, Abd-El Haleem D, Ibrahim A, Awadallah E, Ashmawy I. Paraoxonase-1 gene Q192R and L55M polymorphisms and risk of cardiovascular disease in Egyptian patients with type 2 diabetes mellitus. J Diabetes Metab Disord. 2014;13(1):1–7.
23. Khodaeian M, Enayati S, Tabatabaei-malazy O, Amoli MM. Association between genetic variants and diabetes mellitus in Iranian populations : a systematic review of observational studies. J Diabetes Res. 2015;2015:585917.
24. Wamique M, Ali W, Reddy DH, Vishwakarma P, Waseem M. A case control study on HDL associated PON1 enzyme level in

northern Indian type 2 diabetes mellitus patients. Diabetes Metab Syndr Clin Res Rev. 2018;12(6):843–847.
25. Mccarthy JJ, et al. Polymorphisms of the scavenger receptor class B member 1 are associated with insulin resistance with evidence of gene by sex interaction. J Clin Endocrinol Metab. 2009;94(5): 1789–96.
26. Goodarzynejad H, Boroumand M, Behmanesh M, Ziaee S, Jalali A. The rs5888 single nucleotide polymorphism in scavenger receptor class B type 1 (SCARB1) gene and the risk of premature coronary artery disease: a case-control study. Lipids Health Dis. 2016;15(1): 1–9.
27. Osgood D, Corella D, Demissie S, Cupples LA, Wilson PW, Meigs JB, et al. Genetic variation at the scavenger receptor class B type I gene locus determines plasma lipoprotein concentrations and particle size and interacts with type 2 diabetes: the Framingham study. J Clin Endocrinol Metab. 2003;88(6):2869–79.
28. ArulJothi KN, et al. Molecular analysis of the LDLR gene in coronary artery disease patients from the Indian population. Clin Biochem. 2016;49(9):669–674.
29. Wu D-F, et al. Association of rs5888 SNP in the scavenger receptor class B type 1 gene and serum lipid levels. Lipids Health Dis. 2012;11(1):50.
30. Tetik Vardarlı A, Harman E, Bozok Çetintaş V, et al. Polymorphisms of lipid metabolism enzyme-coding genes in patients with diabetic dyslipidemia. Anatol J Cardiol. 2017;17(4): 313–21.