Scavenger Receptor Class B Type 1 Gene Polymorphism as a Possible Genetic Risk Factor for Insulin resistance

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Abstract

The association between SCARB1 rs74830677 (c.1127 C>T, [P.pro 367 leu] P367L) gene polymorphisms and insulin resistance (IR) has been not been analysed and studied. The clinical relevance of these findings remained uncertain and brings into question the importance of SCARB1 in human physiology. So, we investigated the hypothesis the SCARB1 SNPs in association with the risk of developing insulin resistance. A total of 600 individuals, including 300 T2DM and 300 healthy individuals, were enrolled in the study. Blood samples were collected from each T2DM and healthy individual. CT method for the relative quantitation were used for gene expression level in T2DM and healthy controls. Also, restriction fragment length polymorphism analysis was achieved to detect the incidence of genetic polymorphisms. HOMA resistance index and QUICKI were calculated from fasting plasma glucose and insulin values. In the present study, there was strong association observed between T2DM and SCARB1 rs74830677 gene polymorphism (OR = 1.7, 95% CI 1.18–2.71, P = 0.07). Further, T allele were associated with increase serum insulin and HOMA-IR. Our genotyping data showed that SCARB1 rs7480677 variant is significantly higher in subjects with increased HOMA-IR. Thus, we conclude that SCARB1 polymorphisms may play a crucial role in pathogenesis and susceptibility of insulin resistance thus leads to the development of type 2 diabetes mellitus.

Introduction

The scavenger receptor, class B type 1 (SR-B1), is a multiligand membrane receptor protein that functions as a physiologically relevant HDL receptor whose primary role is to mediate selective uptake or influx of HDL derived cholesteryl esters (CEs) into cells and tissues.[1] 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 lecithin cholesteryl aryl transferase (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 [2][3]. Previous findings suggest controversial results so we assumed that human genetic analysis might provide insight into association of insulin resistance and SCARB1 [4]. Remarkably, genetic variation in mice shows overexpression of SRB1 reduces T2DM despite lower HDL and gene deletion increases T2DM despite Higher HDL [5]. Since insulin resistance is considered as an early cause for the progression of T2DM. The role of SCARB1 polymorphisms in the pathogenesis of insulin resistance can be studied to diagnose the disease prior [6]. Thus, the present study aimed to analyze the SCARB1 polymorphisms to explore their genetic relationship with the development of insulin resistance.

Materials And Methods

In this case-control study all the volunteers had to follow a careful screening program. A total of 603 patients recruited from OPD Medicine, King George's Medical University, Lucknow, India. 20 subjects were omitted from the study due to their personnel reasons and not willing to give consent. Lastly, 300 T2DM subjects and 300 control subjects were employed for this study. The subjects were general population
from Uttar Pradesh, India. T2DM is defined as fasting plasma glucose level FBS $\geq 126$ and HbA1C ($\geq 6.5\%$) level repeatedly. This was defined according to the American diabetes association [7]. Additionally, subjects with a diagnosis of T2DM and a history of at least 2 years of treatment without insulin use were enlisted. Controls were healthy group and cohort of KGMU employees and had no familial history of T2DM. Participants identified with T1DM, heart disease, liver cirrhosis, renal disease gastrointestinal disease, pulmonary disease or cancer were debarred for adjustment of confounding factors. Participants were interviewed using a questionnaire for collecting demographic and laboratory investigations. This study was approved by institutional ethics committee, KGMU. approved the ethical consent. All subject signed the approved informed consent.

Blood sample collection

Total of 3mL of venous blood was withdrawn from each participant into anticoagulant EDTA vials (BD Vacutainer®) for extraction of genomic DNA. The DNA was then stored in TE buffer and divided into aliquots then stored at -20°C for genetic analysis. Further, 2mL of whole blood was centrifuged at 1,500 rpm for 10 min and then separated serum was used for the analysis of biochemical profile and HOMA-IR.

Insulin resistance calculation

Insulin sensitivity was assessed by calculating homeostasis Model Assessment parameter of Insulin Resistance

$$\text{HOMA} = \text{insulin(mU/m) } \times \text{[glucose/22.5]}$$

$$\text{QUICKI} = 1/(\log \text{Fasting Insulin} + \log \text{Fasting Glucose in mg/dL})$$

Patients were considered as insulin resistance when HOMA $\geq 2.6$ (high value indicate insulin resistance) and QUICKI $\leq 0.33$ (low value reflects insulin resistance). Fasting insulin was considered to evaluate IR [8].

DNA amplification and Genotyping

DNA was isolated from freshly drawn blood (leucocytes) gained from EDTA tubes by using salting out method. The SCARB1 gene were amplified by polymerase chain reaction using specific primer designed by using Primer3. Further, the PCR products amplified to identify the genotypes were incubated with specific restriction enzyme as recommended by the manufacturer’s protocol. Genotypes were determined after electrophoresis on agarose gel stained with 0.5 µg/ml ethidium bromide visualized in ultraviolet light.

SCARB1 rs74830677

SCARB1 gene polymorphism was detected by polymerase chain reaction (PCR; Applied Biosystems™ Veriti™) followed by restriction fragment length polymorphism. SCARB1 gene was amplified by the
following PCR conditions: 92° C for 6 minutes, followed by 36 cycles of 92° C for 33 seconds, 61° C for 35 seconds, 68° C for 35 seconds and final elongation at 73° C for 6 minutes with precise SCARB1 rs74830677 gene forward primer 5'-TGCTCCAACCAGGAATCAC-3' and reverse primer 5'-CCATCCTCACTTCCTCAAC-3' (Thermoscientific). Amplification was done with a 13 µL PCR mixture comprising 0.5 µL template DNA, 0.5 µL of both primers and master mixes (Thermo Fisher Scientific Inc.). Enzyme digestion was directed in a 13µL final volume comprising of 1 unit of the SetI enzyme. The reaction was conducted at 37° C overnight and the digested products were separated on 2% agarose gel electrophoresis containing EtBr. The genotypes recognised were categorized according to the presence or absence of the enzyme restriction sites. As a result, the TT genotype was a wild homozygote, the CT genotype was a heterozygote and the CC genotype was a variant homozygote.

Expression analysis of SCARB1

The SCARB1 mRNA levels were assessed in 125 Healthy controls and 125 with T2DM. Isolation of total RNA from peripheral blood was performed by using the mini kit (Qiagen, Valencia, CA). Total RNA (10µg) was converted to cDNA using high-capacity cDNA reverse transcription kit (Catalog number: 4368814, Applied Biosystems™). Real-time PCR was performed in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) using βeta actin gene mRNA was used as a reference standard. Reaction was performed by initial denaturation for 2 min at 50°C and for 10 min at 95°C, followed by 40 cycles of PCR (95°C for 15 s; 60°C for 1 min). Reactions were performed using the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). CT method for the relative expression of results were used for the comparison between genes in T2DM and healthy controls.

Statistical analysis

The differences of all parametric variables were analysed by using student’s t test. Chi square (χ²) test was applied to determine the significance of differences in allele and genotype frequency. Allele and genotype frequencies for SCARB1 were calculated by gene counting method. Analysis of variance (ANOVA) was used to test for variance in parameters between genotypes. Differences were considered significant when p was < 0.05. All statistical analyses were performed using SPSS software version 16.0 (SPSS, Chicago, IL).

Results

Clinical Parameter between type 2 diabetes mellitus cases and controls

This study involved 300 individuals with T2DM and 300 healthy controls. Significantly higher levels of TC, LDL-C, HbA1c, and lower levels of HDL-C were demonstrated in T2DM compared to controls (Table 1). T2DM patients showed significantly higher level of BMI than the controls. On the contrary, HOMA-B and HDL levels were significantly reduced in T2DM patients when compared with controls.

SCARB1 genotype and allele distributions
In SCARB1 rs74830677, the frequencies of CC, CT, and TT genotypes in the case group were 20, 29, and 51% respectively; in the control group, the corresponding frequencies were 27, 35 and 38% respectively. Furthermore, significant association were observed with SCARB1 (Table 2). The present result suggests that SCARB1 rs74830677 increases the susceptibility of T2DM.

Association of SCARB1 genotype with HOMA-IR and lipid parameters

In SCARB1 gene polymorphism, TT genotypes had higher VLDL, LDL levels than the CC genotype, but HDL was decreased in TT genotype than the CC and CT genotypes. Further, CC genotype carriers showed the highest values HOMA-IR and HDL followed by CT and TT genotypes of SCARB1. TT genotype had higher serum insulin levels compare to CC and CT genotypes (Table 3).

Gene expression analysis of SCARB1 gene

Using quantitative RT-PCR transcript levels we compared mRNA levels in T2DM. We observed significantly altered mRNA expression in SCARB1 gene, verifying that the expression of SCARB1 gene at the transcriptional level is altered in T2DM, but not in healthy controls (Fig. 1).

SRB1 and KEGG pathway

Protein-protein interaction plays key role in predicting the protein function of target protein and drug ability of molecules. The majority of genes and proteins realize resulting phenotype functions as a set of interactions. Elucidation of protein interaction networks also contributes greatly to the analysis of signal transduction pathways. Recent developments have also led to the construction of networks having all the protein-protein interactions using computational methods for signaling pathways and protein complex identification in specific diseases [9]. Protein-Protein interaction of SRB1 with APOE and APOB directly influence each other in molecular pathways and SCARB1 positively interacts with 3 proteins. (Fig. 2)

Association of HOMA-IR with serum Insulin and HDL levels

A positive correlation was documented between the insulin serum level and HOMA-IR index ($r = 0.758617163$). The overall population that was studied also demonstrated positive correlation of serum insulin level and the HOMA-IR (Fig. 3)

Discussion

Numerous studies previously showed the association of the SCARB1 gene with T2DM although SRB1 is not an uncommonly studied gene in T2DM, there is a limited understanding of whether these variant is associated with insulin resistance [10]. The data remains controversial due to variations in association with insulin resistance on different ethnic populations. The clinical relevance of these findings remained uncertain and brings into question the importance of SRB1 in human physiology. In the present study we have investigated SCARB1 rs78430677 polymorphism and the development towards risk of insulin resistance.
We also observed higher frequencies of TT genotype and T allele of SCARB1 in T2DM patients as compared to controls and it is significantly associated with T2DM. This result is in agreement with McCarthy et al. (2015)[11]. Further, Pérez-Martínez et al. (2005) [12] who observed an exon 1 polymorphism at the SCARB1 gene is related with significantly different insulin sensitivity in response to changes in the quality of dietary fat. Recently, a study by Himanshu et al. (2022 & 2020) [13][10] detected a strong association between SCARB1 polymorphism and type 2 diabetes mellitus. Moreover, Cerda et al. (2010) suggested that SCARB1 gene polymorphism are related to variation in serum lipids in Brazilian population.

To investigate whether the SCARB1 are associated with insulin resistance, we performed the homeostasis model assessment of insulin resistance for all the study groups. Our results showed that genotypes and alleles of SCARB1 polymorphism were significantly associated with the increased risk of insulin resistance. The HOMA-IR index was significantly increased among T2DM patients with TT genotypes of SCARB1 as compared to CC genotype. It is suggested that genetic variation in the SCARB1 could be a risk factor for insulin resistance and the development of T2DM. This agreed with the results of McCarthy et al. (2015)[2] who found that SCARB1 gene polymorphism was associated with increased risk of insulin resistance in Mexican- American Population. Similarly, we observed a significant association between SCARB1 rs74830677 polymorphism and the risk of insulin resistance.

On the other hand, the present study has some potential limitations, after completion of research, we suggest that the study to be conducted on a large sample size with multicentric considering different ethnic groups to identify the potential relationship of these loci. Studies with a larger sample size in different races will help us to understand the relationship between SCARB1 rs74830677 polymorphism with diabetes mellitus.

In conclusion, SCARB1 polymorphisms may be involved in the pathogenesis of insulin resistance by causing cholesterol metabolism abnormalities and thereby modulating the expression of GLUT-4. A further detailed study with increased number of subjects and genotyping of other polymorphism may help in clarifying the complete role of SCARB1 in insulin resistance and the regulation of GLUT-4.

**Declarations**

**Authors contribution:** Mohd Wamique, Sandeep Pandey carried out the study. Mohd Wamique, Wahid Ali, D Himanshu prepares the manuscript and analysed the data. D Himanshu, Akhilesh Tamrakar, Wahid Ali participated in modifying the manuscript. All authors confirm the final version of the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Research involving human participants and/or animals Animal handling and experimental protocols were approved by institutional ethics committee, King George's Medical University (Ref. Code: 71 ECM II B Thesis/P 13).

**Conflict of Interest** The authors declare no conflict of interest

**References**


**Tables**

Tables 1-3 are available in the supplementary files section.

**Figures**

![Figure 1](image)

Figure 1

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Figure 2

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Figure 3

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Supplementary Files

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- EthicalandstudyPerforma.pdf
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- Table2.docx
- Table3.docx