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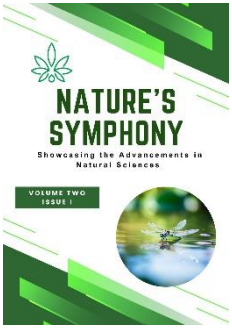

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Research Article

Polymorphic Assessment of the Proprotein Convertase Subtilisin/Kexin Type 9 (*PCSK9*) Variant Rs11591147 in Relation to Coronary Artery Disease in Pakistani Subjects

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ABSTRACT

Coronary artery disease (CAD) is caused by the narrowing of coronary arteries because of plaque formation in response to multiple genetic and environmental factors. Many genetic markers associated with CAD susceptibility have been identified in extensive studies over the past few years. The enzyme proprotein convertase subtilisin/kexin type 9 (PCSK9) targets LDL receptors for degradation and decreases the clearance from the circulatory system of LDL cholesterol (LDLC). Loss-of-function mutations in the *PCSK9* gene increase LDLC clearance, however. One such variant, rs11591147 (also known as G137T or R46L), is protective and more prevalent in White populations compared to other ethnic groups. The present study evaluated the allele and genotype frequencies of rs11591147 and investigated the effect of rs11591147 on CAD risk as well as its relationship with lipid levels in a Pakistani population. KASPar allelic discrimination assay was used to genotype 625 samples (405 CAD cases and 225 controls). Lipid profiles for the study participants were measured through spectrophotometric analysis. In CAD cases, the frequency of the risk allele (G) was slightly higher (0.999) than that in controls (0.995), albeit without a statistically significant difference (p=0.252). No association with CAD risk was observed (p=0.29). However, the SNP showed an impact on lipid levels, with each risk allele increasing LDLC by 14.81 ± 15.8 mg/dL and total cholesterol (TC) by 9.9 ± 30.4 mg/dL (mean ± SE). While this variant did not show a statistically significant association with CAD risk in the study, its effect direction aligns with observations in White populations. Future studies with larger sample sizes from all provinces of Pakistan and analysis of other *PCSK9* variants are recommended to clarify the role of *PCSK9* variants in CAD susceptibility.

INTRODUCTION

Coronary artery disease (CAD) remains the single leading cause of mortality worldwide despite the implementation of prevention and management measures. CAD is a multifactorial disease involving multiple genes that interact with the environment to affect the progression of the disease (Shabana *et al.*, 2018b; Shabana *et al.*, 2020a). The risk factors for CAD are grouped into modifiable and non-modifiable risk factors. The modifiable risk factors include dietary habits/lifestyle factors and physical activity whereas non-modifiable risk factors include age, male sex, family history of CAD etc. (Shahid *et al.*, 2017a). In Pakistan, a high

proportion of blood marriages results in the concentration of risk alleles/genotypes for many genetic diseases (Chauhan *et al.*, 2020; Sarwar *et al.*, 2023). The increased genetic susceptibility together with reduced physical activity and a high fat diet results in a continuous increase in the number of CAD cases diagnosed per year (Shahzadi *et al.*, 2016; Shabana *et al.*, 2020b; Shahid *et al.*, 2020).

Proprotein convertase subtilisin/kexin type 9 (*PCSK9*, OMIM 607786) consists of 12 exons and is a highly polymorphic gene (Ezzat *et al.*, 2021). The gene encodes a polypeptide consisting of 692 amino acids residues(Cesaro *et al.*, 2020). It is a member of the subtilisin family of kexin like proconvertases and promotes the degradation of low-density lipoprotein receptors (LDLRs), the primary sites for low-density lipoprotein cholesterol (LDLC) clearance from the peripheral circulation. PCSK9 is a glycoprotein that is highly expressed in liver, intestine and kidneys (Seidah *et al.*, 2003). Like other proconvertases, this protein contains a signal sequence (amino acids 1 to 30), a pro-domain, a catalytic domain and a C- terminal domain that is rich in cysteine residues (Dobó *et al.*, 2022). PCSK9 directs the degradation of LDLRs by targeting them to lysosomes for degradation and prevents them from recycling to the cell surface leading to decreased LDLC clearance and increased LDLC concentration. In a mouse model, overexpression of *PCSK9* resulted in a reduction in LDLR in liver and a corresponding increase in circulating LDLC (Lalanne *et al.*, 2005; Poznyak *et al.*, 2023). Similarly mice lacking *PCSK9* had higher LDLR and lower circulating LDLC (Rashid *et al.*, 2005).

Loss-of-function mutations in *PCSK9* are common and result in a decrease in the LDLC concentration because of increased expression of LDRs thereby lowering CAD risk (Libby and Tokgözoğlu, 2022). Gain-of-function mutations are rare and are reported to be associated with an increase in LDLC levels (Abifadel *et al.*, 2003). The polymorphism *PCSK9* rs11591147 in exon 1 is a G>T transversion at position 137 resulting in the replacement of arginine with leucine at amino acid 46 (G137T or R46L) (Soko *et al.*, 2016b). This polymorphism is more common in Whites (3.2%) than in Black populations (0.6%) (Kotowski *et al.*, 2006). R46L is a loss-of-function polymorphism and reduces LDL-C by 0.28 to 0.54 mmol/l (9% to 15%), with a CAD risk reduction up to 47% in carriers versus noncarriers (Cohen *et al.*, 2006; Scartezini *et al.*, 2007). The ancestral allele 'G' is risk allele and mutant allele is protective.

The Pakistani population represents a unique ancestry from Asia on the basis of distinct religious, social and cultural practices (Wall *et al.*, 2023). The increasing prevalence of CAD in Pakistan in recent decades and unique genetic architecture of the Pakistani population necessitate the study of genetic markers to provide a baseline information for the implementation of clinical modalities. We therefore aimed to genotype the rs11591147 SNP of the *PCSK9* gene in a cohort of Pakistani people and determine its interaction with various lipid parameters.

2. MATERIAL AND METHODS

2.1 Study Participant Recruitment

For the current study 405 CAD patients and 220 healthy controls were included. The inclusion and exclusion criteria have been described previously (Shahid *et al.*, 2016; Shahid *et al.*, 2017a; Shahid *et al.*, 2017b; Shabana *et al.*, 2018b; Shabana *et al.*, 2020a). The patients were diagnosed with nonfatal myocardial infarction by consultant cardiologists based on ECG, echocardiogram, angiography, troponin T/I and clinical history.

Controls consisted of healthy subjects with no family history of CAD, not on lipid lowering medication and stayed disease free for long time (mean age of control group is 56 ± 10.5) Study subjects were of Pakistani origin for up to three generations. All subjects gave a written informed consent. The study was approved by the departmental ethical committee of University of the Punjab, Lahore, and all the procedures were in compliance with the Helsinki declaration.

2.2 Blood Sampling and Biochemical Analyses

Blood samples were collected after 8-12 hr of fasting in EDTA and clot-activator gel vacutainers (2ml each). The samples in EDTA vials were used for DNA isolation while the gel clot activator containing vacutainers were centrifuged and the serum was collected in sterilized Eppendorf tubes. Infectious agents (HBV, HCV, HIV) were screened from the samples; those tested positive for infectious pathogens were discarded. Serum lipid profile (TC, TG, HDLC, and LDLC) was determined calorimetrically using commercially available kits (Spectrum Diagnostics, Egypt). Optical density was measured using an Epoch Biotek microplate reader (Biotek instruments, Highland Park).

2.3 Genotyping of rs11591147

2.3.1 DNA Extraction and Quantification

Genomic DNA was extracted from blood leukocytes using the Wizard® Genomic DNA Purification Kit (Promega, USA). DNA quantification was performed with a Nanodrop ND-8000 spectrophotometer (USA), and all samples were adjusted to a concentration of 5 ng/µL. The DNA samples were then spotted onto 384-well MicroAmp plates using a Biomerk FX robotic liquid handling system (Beckman Coulter).

2.3.2 Genotyping Procedure

Genotyping of the *PCSK9* rs11591147 variant was conducted using the KASPar allelic discrimination technique. The KASPar SNP assay includes two allele-specific forward primers (one for each allele) with unique unlabelled sequences at the 5′ end, a shared reverse primer, and two fluorophore-labelled oligonucleotides, labelled with FAM and VIC at the 5′ end, along with two 3′ quencher-labelled oligonucleotides complementary to the fluorophores (Supplementary Table 1). The KASPar master mix was prepared with a hot-start DNA polymerase, dNTPs, ROX dye, 100 µM of each allele-specific primer, a common reverse primer, and 10 mM Tris-HCl. The master mix and SNP assay were thawed from -20°C, gently mixed, briefly centrifuged, and kept on ice. A reaction mix was made by combining 900 µL of KASPar master mix, 26.4 µL of SNP assay, and 900 µL of Sigma water, and 4 µL of this mix was added to each well containing the DNA samples, with non-template controls (NTCs) included. After confirming the reaction mixture in all wells, the plate was sealed, centrifuged, and placed in a thermal cycler (BioRad™) with a thermal pad to prevent evaporation. The PCR conditions involved initial enzyme activation at 94°C for 15 minutes, followed by 10 cycles of 94°C for 20 seconds and annealing/extension from 65°C to 57°C (decreasing 0.8°C per cycle), and 26 cycles of 94°C for 20 seconds with 57°C for 1 minute. After cooling for 30 minutes, the plate was read on a FRET-based sequence detection system (ABI PRISM 7900HT). Genotypes were confirmed by conventional DNA sequencing for 10 samples. Known heterozygous controls were included in each plate.

2.4 Statistical Analysis

Data analysis was conducted using SPSS (IBM, version 22). Independent sample t-tests compared continuous variables (lipid levels)

Table 1. Anthropometric and biochemical parameters of study subjects

Variables	Cases	Controls	*p*-value
Number	405	220	-
Age (years)	59.1±12.6	56 ± 10.5	0.002
Sex Males (n) Females (n)	 216 189	 120 100	0.27
BMI (Kg/m^2)	22.46±6.75	21.46±9.11	0.119
Diabetes (%)	64.6	13.6	$5.1x10^{-34}$
Hypertension (%)	62.1	16.4	$8.9x10^{-28}$
Smoking (%)	29.5	10.5	$7.3x10^{-08}$
TC	207.5±53.7	175.4±43	$8.8x10^{-14}$
TG	212.4±70	188±66.3	$2.6x10^{-5}$
LDLC	106±28.9	84.7±17	$6.3x10^{-22}$
HDLC	45.2±11.9	67.4±16.3	$1.8x10^{-66}$

Categorical variables are expressed in number with percentage. Continuous variables are expressed as mean±SD. TC: total cholesterol (reference value <200mg/dl), TG: triglycerides (reference value <150mg/dl), LDLC: low density lipoprotein cholesterol (reference value <70mg/dl), HDLC: high density lipoprotein cholesterol (reference value >60mg/dl), BMI: Body Mass Index. *p*-value is the level of statistical significance

between CAD cases and controls. Hardy-Weinberg equilibrium was assessed with a χ^2 goodness-of-fit test. The association between the SNP and CAD (a binary outcome) was analyzed using binary logistic regression, and the effect of the *PCSK9* variant on lipid levels was assessed by linear regression.

3. RESULTS

3.1 Characteristics of Study Participants

Table 1 provides an overview of the baseline biochemical and anthropometric data of the study participants. The case group included a higher percentage of individuals with diabetes, hypertension, and a greater prevalence of smokers compared to the control group. Levels of total cholesterol (TC), triglycerides (TG), and LDL cholesterol (LDLC) were significantly elevated in the case group, whereas HDL cholesterol (HDLC) was lower compared to the controls. There was no significant difference in body mass index (BMI) between the two groups.

3.2 Allele and Genotype Frequencies of the *PCSK9* R46L Variant (rs11591147)

Distinct clusters were observed, with only known heterozygotes clearly separated from the risk homozygotes (Table 2). Ten samples across the plates did not align distinctly with either the homozygote or heterozygote clusters (see Supplementary Figure 1). Direct sequencing confirmed that 3 of these samples were heterozygous, as shown in Supplementary Figure 2. The SNP demonstrated a 98% call rate and was in Hardy-Weinberg equilibrium for both cases and controls, as well as the combined sample group. Although the risk allele (G) frequency was slightly higher in cases (0.999) compared to controls (0.995), this difference was not statistically significant (p=0.252). The variant showed no significant association with CAD risk (p=0.29) (Table 3). The average increase in LDLC per risk allele was 14.81 ± 15.8 mg/dL, and the increase in TC per risk allele was 9.9 ± 30.4 mg/dL, though these effects were not statistically significant, as presented in Table 3.

Table 2. Frequency of *PCSK9* rs11591147 polymorphism

Gene	SNP	(HWE) *p*			RAFs		
		Cases	Controls	Total	Cases	Controls	*p*
PCSK9	rs11591147	0.98	0.95	0.95	0.999	0.995	0.252

HWE *p* is Hardy Weinberg p-value, RAF is risk allele frequency, *p* is statistical significance level

Table 3. Association of rs11591147 with CAD, LDL-C and TC

SNP	CAD (OR)	C.I. (*p value)*	β LDLC±Se (*p value)*	β TC±Se (*p value)*
rs11591147	3.71	0.34-41.2 (0.29)	14.81±15.8 (0.35)	9.9±30.4 (0.74)

OR is Odds ratio, C.I. is confidence interval, TC is total cholesterol level, β is the mean increase (standard error) in LDL-C and TC levels. The association of rs11591147 with CAD is shown as Odds ratio as analysed by binary logistic regression. Per allele effect size (β) on serum LDLC and TC is shown as mg/dl ± Standard error

4. DISCUSSION

The genetic factors underlying coronary artery disease (CAD) are complex and not fully understood, though numerous candidate markers and findings from genome-wide association studies (GWAS) have emerged in recent years. It is crucial to validate these genetic markers across different ethnic groups to develop targeted preventive strategies. The Pakistani population, with distinct cultural practices such as a high prevalence of consanguineous marriages, offers a unique genetic pool where specific alleles and genotypes are more concentrated, often leading to increased occurrences of genetic disorders. Against this backdrop, our study explored the association between the *PCSK9* rs11591147 variant and CAD risk, aiming to identify its potential influence on lipid profile parameters within a case-control design. CAD patients exhibited an altered lipid profile characterized by elevated levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDLC), triglycerides (TG), and reduced high-density lipoprotein cholesterol (HDLC). Low HDLC, a key feature of dyslipidemia, is associated with an increased CAD risk, particularly when TG levels are moderately elevated, creating a classic CAD risk profile. For men, HDLC levels below 40 mg/dL, and for women below 45 mg/dL, increase CAD risk. Moreover, a fasting TG level above 150 mg/dL is another CAD marker. Hypercholesterolemia, whether genetic or acquired, also poses a significant risk, with estimates indicating it may contribute to 56% of heart disease cases (Tsai *et al.*, 2014).

In multifactorial conditions like CAD, many SNPs with varying degrees of impact collectively influence disease risk. The effect of each variant on CAD outcome can be modified by environmental factors and other genetic variations within the same or nearby genes or pathways. No single variant is essential or solely sufficient to determine disease risk. The contribution of each SNP to CAD is determined by both the frequency of the variant (minor allele frequency) and the associated relative risk it confers (Cameron *et al.*, 2006; Zhao *et al.*, 2006).

In our analysis of the *PCSK9* R46L polymorphism, we found that the 46L allele did not significantly correlate with CAD in the

Pakistani cohort, aligning with findings from a UK study on healthy middle-aged men (Soko *et al.*, 2016a). However, in the UK study, the 46L allele was linked to increased LDLC and TC levels, a pattern not observed in our study. In contrast, research on African American and Native American populations has shown that the R46 allele is associated with reduced LDLC and a marked decrease in CAD risk (Talmud *et al.*, 2002; Maxwell and Breslow, 2004; Maxwell *et al.*, 2005). Although the exact mechanism by which the R46L variant operates remains unclear, it is hypothesized that loss-of-function mutations may disrupt the synthesis or cellular trafficking of *PCSK9*, reducing its secretion. The amino acid substitution from a polar arginine (R46) to a hydrophobic leucine (46L) could affect the protein's function by altering the interaction between the N-terminal pro-domain and the catalytic C-domain. In one mechanism, PCSK9 binds to LDL receptors in the Golgi and directs them to lysosomes for degradation, impacting LDLC levels. Another mechanism suggests PCSK9 binds surface LDL receptors and promotes their degradation within lysosomes (Talmud *et al.*, 2002; Tan *et al.*, 2012).

Pakistan, with a population exceeding 235 million, has a high prevalence of CAD and its risk factors, which surpasses global rates. Contributing factors include high rates of consanguinity, limited health literacy, high-fat diets, sedentary lifestyles, and low socioeconomic conditions, particularly in rural areas, which make up over 70% of the population. Identifying genetic risk factors is crucial for developing affordable, preventive healthcare options for a population with limited access to healthcare services (Shabana *et al.*, 2018a; Shabana and Hasnain, 2018).

Several limitations were noted, including a limited sample size primarily from the Punjab province and the examination of only one *PCSK9* variant. Although the study's power exceeded 90% with 625 samples, larger sample sizes are necessary to gain a more comprehensive understanding of the gene-disease relationship. Recruitment challenges persist in Pakistan due to limited immediate benefits for participants and difficulties in conveying research significance. Another limitation is the significant lipid profile differences between cases and controls, potentially introducing bias. Prevalent risk factors like diabetes and hypertension in the control group may also have influenced the results, though adjustments were made to account for these factors. The cross-sectional design restricts the study to a single-point analysis, meaning that changes due to lipid-lowering treatments in cases or disease progression in controls were not tracked. Nonetheless, this design provides insight into the relationship direction between risk factors and disease outcomes. Increasing the sample size would further reduce potential false-positive results. Further research should investigate gene-environment interactions and consider samples from other Pakistani provinces, as well as additional *PCSK9* variants, to enhance understanding of CAD genetics.

5. CONCLUSION

Although the SNP interaction with CAD could not be clearly established in the current study, the direction of the effect was similar to that in White people i.e., the minor allele appeared to be a protective allele, and the major allele was the risk allele with a slightly higher frequency in the cases than in the controls. The effect appeared to be mediated through a change in serum TC and LDLC levels. These findings, however, need to be replicated in larger cohorts in the future to establish a clear relationship between PCSK9 variants and CAD.

Ethics Approval and Consent to Participate

The study was approved by the institutional ethics committee and all procedures were

carried out in compliance with the Helsinki Declaration.

Availability of Data and Materials

All the necessary information has been provided along with the manuscript, however, the corresponding author can be contacted for any information related to this paper.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Author Contributions

SUS and Shabana conceived the study concept, SUS carried out the bench work, SUS, SS and Shabana analyzed the results, and drafted the manuscript; and SH critically reviewed the manuscript and supervised the study.

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REFERENCES

Abifadel, M., Varret, M., Rabès, J.-P., Allard, D., Ouguerram, K., Devillers, M., Cruaud, C., Benjannet, S., Wickham, L. and Erlich, D. 2003. Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia. *Nature Genetics*, 34:154-156.

Cameron, J., Holla, Ø. L., Ranheim, T., Kulseth, M. A., Berge, K. E. and Leren, T. P. 2006. Effect of mutations in the *PCSK9* gene on the cell surface LDL receptors. *Human Molecular Genetics*, 15:1551-1558.

Cesaro, A., Bianconi, V., Gragnano, F., Moscarella, E., Fimiani, F., Monda, E., Scudiero, O., Limongelli, G., Pirro, M. and Calabrò, P. 2020. Beyond cholesterol metabolism: the pleiotropic effects of proprotein convertase subtilisin/kexin type 9 (PCSK9). . *Biofactors*, 46:367-380.

Chauhan, B. G., Yadav, D. and Jungari, S. 2020. Association between consanguineous marriage and child nutritional outcomes among currently married women in Pakistan. *Clinical Epidemiology and Global Health*, 8:38-44.

Cohen, C, J., Boerwinkle, E., Mosley Jr, T. H. and Hobbs, H. H. 2006. Sequence variations in *PCSK9*, low LDL, and protection against coronary heart disease. *New England Journal of Medicine*, 354:1264-1272.

Dobó, J., Kocsis, A., Dani, R. and Gál, P. 2022. Proprotein convertases and the complement system. *Frontiers in Immunology*, 13:958121.

Ezzat, G. M., Nasreldein, A., Mohamed, G. A., Elsewify, W. A. and Dahpy, M. A. 2021. The link between proprotein convertase subtilisin/kexin 9 serum levels and *E670G* gene polymorphism and the risk of ischemic stroke in patients of the south of Egypt. *Journal of Current Medical Research and Practice*, 6:200-207.

Kotowski, I. K., Pertsemlidis, A., Luke, A., Cooper, R. S., Vega, G. L., Cohen, J. C. and Hobbs, H. H. 2006. A spectrum of *PCSK9* alleles contributes to plasma levels of low-density lipoprotein cholesterol. *The American Journal of Human Genetics*, 78:410-422.

Lalanne, F., Lambert, G., Amar, M. J., Chétiveaux, M., Zaïr, Y., Jarnoux, A.-L., Ouguerram, K., Friburg, J., Seidah, N. G. and Brewer, H. B. 2005. Wild-type *PCSK9* inhibits LDL clearance but does not affect

apoB-containing lipoprotein production in mouse and cultured cells. *Journal of Lipid Research*, 46:1312-1319.

Libby, P. and Tokgözoğlu, L. 2022. Chasing LDL cholesterol to the bottom—PCSK9 in perspective. *Nature Cardiovascular Research*, 1:554-561.

Maxwell, K. N. and Breslow, J. L. 2004. Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. *Proceedings of the National Academy of Sciences of the United States of America*, 101:7100-7105.

Maxwell, K. N., Fisher, E. A. and Breslow, J. L. 2005. Overexpression of PCSK9 accelerates the degradation of the LDLR in a post-endoplasmic reticulum compartment. *Proceedings of the National Academy of Sciences of the United States of America*, 102:2069-2074.

Poznyak, A. V., Sukhorukov, V. N., Eremin, I. I., Nadelyaeva, I. I., Gutyrchik, N. A. and Orekhov, A. N. 2023. Proprotein convertase subtilisin/kexin 9 as a modifier of lipid metabolism in atherosclerosis. *Biomedicines*, 11:503.

Rashid, S., Curtis, D. E., Garuti, R., Anderson, N. N., Bashmakov, Y., Ho, Y., Hammer, R. E., Moon, Y.-A. and Horton, J. D. 2005. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking *PCSK9*. *Proceedings of the National Academy of Sciences of the United States of America*, 102:5374-5379.

Sarwar, S., Shabana, Sajjad, K. and Hasnain, S. 2023. Genetic studies in the Pakistani population reveal novel associations with ventricular septal defects (VSDs). *BMC pediatrics*, 23:67.

Scartezini, M., Hubbart, C., Whittall, R., Cooper, J., Neil, A. and Humphries, S. 2007. The *PCSK9* gene R46L variant is associated with lower plasma lipid levels and cardiovascular risk in healthy UK men. *Clinical Science*, 113:435-441.

Seidah, N. G., Benjannet, S., Wickham, L., Marcinkiewicz, J., Jasmin, S. B., Stifani, S., Basak, A., Prat, A. and Chrétien, M. 2003. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proceedings of the National Academy of Sciences*, 100:928-933.

Shabana, Shahid, S. U. and Hasnain, S. 2018a. Use of a gene score of multiple low-modest effect size variants can predict the risk of obesity better than the individual SNPs. *Lipids in health and disease*, 17:1-9.

Shabana, Shahid, S. U. and Sarwar, S. 2020a. The abnormal lipid profile in obesity and coronary heart disease (CHD) in Pakistani subjects. *Lipids in health and disease*, 19:1-7.

Shabana, N., Ashiq, S., Ijaz, A., Khalid, F., ul Saadat, I., Khan, K., Sarwar, S. and Shahid, S. U. 2018b. Genetic risk score (GRS) constructed from polymorphisms in the *PON1*, *IL-6*, *ITGB3*, and *ALDH2* genes is associated with the risk of coronary artery disease in Pakistani subjects. *Lipids in health and disease*, 17:224.

Shabana, N., Shahid, S. U. and Irfan, U. 2020b. Genetic contribution to congenital heart disease (CHD). *Pediatric cardiology*, 41:12-23.

Shabana, S., Saleem Ullah and Hasnain, S. 2018. Identification of genetic basis of obesity and mechanistic link of genes and lipids in Pakistani population. *Bioscience reports*, 38:BSR20180281.

Shahid, S. U., Cooper, J. A., Beaney, K. E., Li, K., Rehman, A. and Humphries, S. E. 2017a. Genetic risk analysis of coronary artery disease in Pakistani subjects using a genetic risk score of 21 variants. *Atherosclerosis*, 258:1-7.

Shahid, S. U., Cooper, J. A., Rehman, A. and Humphries, S. E. 2016. Association of *ACE* and *NOS3* gene polymorphism with blood pressure in a case control study of coronary artery disease in Punjab, Pakistan. *Pakistan Journal of Zoology*, 48.

Shahid, S. U., Shabana and Rehman, A. 2017b. Association patterns of endothelial nitric oxide synthase gene (*NOS3*) variant *Glu298Asp* with blood pressure and serum lipid levels in subjects with coronary artery disease from pakistan. *Annals of human genetics*, 81:129-134.

Shahid, S. U., Shabana and Rehman, A. 2020. Predictive value of plasma lipid levels for coronary artery disease (CAD). *Biologia*, 75:1455-1463.

Shahzadi, S., Chaudhry, M., Arooj, I. and Hasnain, S. 2016. A single-nucleotide polymorphism in C12orf43 region is associated with the risk of coronary artery disease in a Pakistani cohort. *Biochemical genetics*, 54:676-684.

Soko, N. D., Masimirembwa, C. and Dandara, C. 2016a. Pharmacogenomics of Rosuvastatin: A glocal (global+ local) African perspective and expert review on a statin drug. *OMICS: A Journal of Integrative Biology*, 20:498-509.

Soko, N. D., Masimirembwa, C. and Dandara, C. 2016b. Pharmacogenomics of rRosuvastatin: A glocal (global+ local) African perspective and expert review on a statin drug. *OMICS: A Journal of Integrative Biology*, 20:498-509.

Talmud, P. J., Hawe, E., Martin, S., Olivier, M., Miller, G. J., Rubin, E. M., Pennacchio, L. A. and Humphries, S. E. 2002. Relative contribution of variation within the *APOC3*/*A4*/*A5* gene cluster in determining plasma triglycerides. *Human Molecular Genetics*, 11:3039-3046.

Tan, A., Sun, J., Xia, N., Qin, X., Hu, Y., Zhang, S., Tao, S., Gao, Y., Yang, X. and Zhang, H. 2012. A genome-wide association and gene–environment interaction study for serum triglycerides levels in a healthy Chinese male population. *Human Molecular Genetics*, 21:1658-1664.

Tsai, C.-W., North, K. E., Tin, A., Haack, K., Franceschini, N., Saroja Voruganti, V., Laston, S., Zhang, Y., Best, L. G. and MacCluer, J. W. 2014. Both rare and common variants in PCSK9 influence plasma low-density lipoprotein cholesterol level in American Indians. *The Journal of Clinical Endocrinology & Metabolism*, 100:E345-E349.

Wall, J. D., Sathirapongsasuti, J. F., Gupta, R., Rasheed, A., Venkatesan, R., Belsare, S., Menon, R., Phalke, S., Mittal, A. and Fang, J. 2023. South Asian medical cohorts reveal strong founder effects and high rates of homozygosity. *Nature Communications*, 14:3377.

Zhao, Z., Tuakli-Wosornu, Y., Lagace, T. A., Kinch, L., Grishin, N. V., Horton, J. D., Cohen, J. C. and Hobbs, H. H. 2006. Molecular characterization of loss-of-function mutations in *PCSK9* and identification of a compound heterozygote. *The American Journal of Human Genetics*, 79:514-523.