Pakistan J. Zool., vol. 50(6), pp 2295-2300, 2018. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2018.50.6.2295.2300

Association of Loci 6q25.1/rs6922269 and 9p21.3/rs1333049 with Risk of Coronary Heart Disease in Patients Visiting Hospitals of Gujranwala and Lahore Divisions

Iqra Arooj[1,*] and Shahida Hasnain[2]

[1]*Department of Microbiology and Molecular Genetics, The Women University, Multan*
[2]*Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore*

Article Information
Received 25 April 2018
Revised 18 May 2018
Accepted 28 May 2018
Available online 11 October 2018

Authors' Contributions
SH designed the study, helped in statistical analysis and supervised the study. IA performed experimental work, statistically analyzed the data and wrote the article.

Key words
Coronary heart disease, 6q25.1, 9p21.3, rs6922269, rs1333049.

ABSTRACT

Cardiovascular diseases such as coronary heart disease (CHD) are a growing health concern in developing and under-developed countries. Some of their risk factors are smoking, obesity, diabetes, family history, high blood pressure etc. Previous genome wide association studies have identified association of large number of loci with risk of CHD in different populations. The purpose of this study was to determine the association of 6q25.1/rs6922269 and 9p21.3/rs1333049 loci with risk of CHD in a population of Punjab province of Pakistan. A total of 645 subjects were included in this study of which 435 were patients visiting hospitals of Lahore and Gujranwala districts. All the subjects were genotyped by allele-specific PCR. It was observed that the loci 6q25.1/rs6922269 and 9p21.3/rs1333049 were significantly associated with risk of CHD in this population (P=0.001 and 0.012, respectively).

INTRODUCTION

Cardiovascular diseases including coronary heart disease (CHD) are the leading cause of mortality worldwide and are responsible for approximately one in three deaths across the globe (Mensah *et al*., 2013). Several risk factors are associated with these diseases including metabolic syndrome, for instance (Saqlain *et al*., 2018). It has been estimated that one in five middle-aged adults may have underlying CHD in urban areas of Pakistan (Gaziano *et al*., 2010; Ahmed *et al*., 2013). Candidate gene association studies have identified numerous genes responsible for the pathogenesis of CHD (Thompson and Danesh, 2006), but this approach has not proved highly productive due to the fact that only a few of the identified genes demonstrate association in replication studies (Hirschhorn *et al*., 2002). More recently, genome-wide association studies emerged as a strong approach for identifying previously unknown risk loci. First report of an association between a polymorphism in MTHFD1L gene and CHD surfaced in 2007 when meta-analysis of two large genome-wide association studies found a statistically significant association between rs6922269 and CHD (Samani *et al*., 2007). Subsequent reports in this replicated in some of the succeeding studies, while in others no significant association was observed between rs6922269 and CHD or related disease states (Muendlein *et al*., 2009; Angelakopoulou *et al*., 2011; Hubacek *et al*., 2016). rs6922269 was also described for being significantly protective against myocardial infarction (Saade *et al*., 2011). In addition to CHD, polymorphisms in MTHFD1L gene have been found to be important in the pathogenesis of several other human diseases including cancer, Alzheimer's disease, and neural tube abnormalities (Parle-McDermott *et al*., 2009; Ren *et al*., 2011; Johnson *et al*., 2016). Consistent association of a 58kb interval on human chromosomal location 9p21 with CHD proved impulsive for its identification in a genome wide association scan (McPherson *et al*., 2007). Subsequent identification and investigation of genetic variants present in this region revealed that a number of single nucleotide polymorphisms (SNPs) and haplotypes located here were significantly associated with the risk of CHD and some other diseases but the most powerful association with CHD was exhibited by rs1333049 out of all the SNPs that were present at chromosome 9p21.3 (WTCCC, 2007). The same genetic variant was later found to be associated with several other diseases including stroke, peripheral artery disease, abdominal aortic aneurysm, gout, Alzheimer's disease and endometriosis (Cluett *et al*., 2009; Karvanen *et al*., 2009; Yu *et al*., 2010; Pagliardini *et al*., 2013; Wei *et al*., 2014; Zheng *et al*., 2016). Previous studies have also identified

* Corresponding author: iqra.6051@wum.edu.pk
0030-9923/2018/0006-2295 $ 9.00/0

9p21.3/rs1333049 as risk locus for CAD or MI in Asian populations including Pakistani population (Saleheen *et al*., 2010). Replication of association between rs1333049 and risk of CHD in other studies has contributed towards establishment of this locus as a risk factor for CHD which has provided a new hope to decipher the genetic basis of complex diseases (Hinohara *et al*., 2008). However, the direct mechanistic link between the loci 6q25.1 and 9p21.3, and atherosclerosis is yet to be determined. In this study, we sought to replicate the association of loci 6q25.1/rs6922269 and 9p21.3/rs1333049 with CHD in Pakistani patients visiting hospitals of Gujranwala and Lahore districts representing Central Punjab.

MATERIALS AND METHODS

CHD cases were defined as having a prior history of myocardial infarction (MI) and/or coronary revascularization which was indicated by the cardiac specialists based on available clinical information and medical records. Controls were healthy volunteers having no diagnosis or symptoms of CHD or any other cardiac disease. The controls were selected based on self-report about their previous medical history and records. Individuals with history of any bacterial or viral infection from last 15 days were excluded. This case–control study was approved by Board of Advance Studies and Ethical Committee of the University of the Punjab. Permission for sample collection was granted by the relevant institutional ethical committees and review boards of the hospitals. All cases and controls provided written or verbal informed consent (if unable to write due to educational background) or consent provided by a family member. The present study included 435 CHD cases, recruited from various clinical settings of Lahore (Punjab Institute of Cardiology, Mayo Hospital, Jinnah Hospital) and Gujranwala (Siddique Sadiq Memorial Trust Hospital, District Headquarters Hospital, Cheema Heart Complex, Social Security Hospital) divisions of Punjab from March to December 2010. There were 210 healthy controls matching to CHD cases in age and gender and comprising hospital employees, employees of government institutions, friends and colleagues of study-staff, attendants of other patients, and manual laborers. They were recruited from the same communities as cases and fulfilled inclusion criteria. Information on co-morbidities was recorded on a pre-designed form for CHD cases and controls. This included diabetes, hypertension and obesity based on clinician's diagnosis and medical record review. Smoking status was also recorded based on the self-reported history. Body mass index (BMI) was calculated as Kg/m^2 from weight measured in kilograms (Kg) and height measured in meters (m). Five ml of blood was drawn from study participants by venipuncture and stored in EDTA vial for later genetic analysis. Isolated serum from the 3 ml blood stored in plain tube was screened for HIV, HCV and HbsAg using one-step device (Acu-check®) and following the manufacturer's instructions.

For SNP genotyping, genomic DNA extraction for CHD cases and controls was performed using the Promega Genomic DNA Purification Kit (Cat # A1125). Allele-specific primers were designed for rs6922269, (forward primer 1 for wild-type allele: 5'-CTG TAA CTG CCA ATA AG-3', forward primer 2 for variant allele: 5'-CTG TAA CTG CCA ATA AA-3', common reverse primer: 5'-CTT CAT GAT AAG CAT TCT-3') and rs1333049 (common forward primer: 5'-GCC ATG TCA GGG CCA GAA GTC G-3', reverse primer 1 for wild-type allele 1: 5'- CCT CTG CGA GTG GCT GCT TTT C-3', reverse primer 2 for variant allele: 5'- CCT CTG CGA GTG GCT GCT TTT G-3') polymorphisms using web-based tool primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/) and synthesized from Gene Link, USA. The PCR thermal cycling programme for rs6922269 consisted of initial denaturation at 94°C for 2 min followed by 33 cycles each of denaturation at 94°C for 1 min, annealing at 54 °C for wild-type allele and 56.5°C for variant allele for 1 min and extension at 72°C for 1 min and final extension at 72°C for 8 min. The PCR thermal cycling programme for rs1333049 comprised of initial denaturation at 95°C for 3 min followed by 35 cycles each of denaturation at 95°C for 1 min, annealing at 63 °C for 50 sec for wild-type allele and 65°C for 1 min for variant allele and extension at 72°C for 1 min and final extension at 72°C for 10 min. The product size was 502bp for rs6922269 polymorphism and 801bp for rs1333049 polymorphism. Descriptive statistics including mean and standard error of mean (SEM) was done in Microsoft Excel. For comparison between case and control groups, student's t-test for unpaired samples was performed. Statistical significance based on t-test was documented using a *P*-value of less than 0.05. Odds ratio and confidence intervals were calculated with the help of online software (http://www.medcalc.org/calc/odds_ratio.php). Calculation of Hardy-Weinberg equilibrium was done by chi square test using a statistical package SPSS version 20.0 (SPSS, USA).

RESULTS

Out of 645 (435 cases and 210 controls) participants, 32 (5.0%) tested positive for HCV and 7 (1.0%) for HbsAg. However, none of the participants tested positive for HIV. The HCV^+ and $HbsAg^+$ samples were excluded from

Table I.- Distribution of 6q25.1/rs6922269 and 9p21.3/rs1333049 genotypes in CHD cases and controls.

Polymorphism	Genotype	Cases (n=403)	Controls (n=203)	OR (95% CI)	Pearson's Chi-squared test p (χ^2)
rs6922269	GG	260	155	0.56 (0.384-0.826)	p=0.012 (8.817)
	GA	127	42	1.76 (1.183-2.630)	
	AA	16	6	1.36 (0.523-3.523)	
rs1333049	GG	73	61	0.52 (0.348-0.763)	p=0.001 (13.231)
	GC	214	102	1.12 (0.800-1.571)	
	CC	116	40	1.65 (1.096-2.476)	

OR, odds ratio; CI, confidence interval.

subsequent analysis. The baseline characteristics of cases and controls are summarized in Supplementary Table I. In the present study, 403 CHD cases (mean age ± SEM years, 55.08 ± 0.535; 77.9% males) and 203 controls (mean age ± SEM years, 51.77 ± 0.421; 77.8% males) were included after screening. Cases were categorized into three groups based on first cardiac event experienced *i.e.* myocardial infarction (MI, N=259; 64.3%), percutaneous coronary intervention (PCI, N=93; 23.1%) and coronary artery bypass grafting (CABG, N=51; 12.6%). Cases were matched to controls on gender (*P*=0.981) and age (*P*=0.565); age at first event for cases was matched with age at recruitment for controls. Systolic blood pressure (SBP) was found to be higher in cases compared to controls (119.44 ± 0.99 vs 114.23 ± 0.48; *P*=0.001) although the average values were within the normal range for both groups. CHD cases and controls were significantly different on pulse rate (*P*=0.008) and smoking ($P=5.374\times10^{-07}$). There were more diabetic and hypertensive CHD cases than controls (diabetic CHD cases vs. controls, 32% vs. 18%: hypertensive CHD cases vs. controls, 39% vs. 27%). As expected, both groups were significantly different based on the family history of CHD or any other CVD ($P=2.087\times10^{-32}$). Supplementary Figure S1 illustrates the genotyping results for rs6922269 and rs1333049 based on allele-specific PCR. In our study, the genotype distributions in CHD cases and in controls were in Hardy-Weinberg equilibrium for rs6922269 (*P*=0.921; *P*=0.143, respectively) as well as rs1333049 (*P*=0.136; *P*=0.822, respectively). The present study showed that association with the risk of CHD was significant for the loci 6q25.1/rs6922269 (OR=1.36; 95% CI=0.52-3.52; *P*=0.012) and 9p21.3/rs1333049 (OR=1.65; 95% CI=1.09-2.47; *P*=0.001) in Pakistani population (Table I).

DISCUSSION

In the present study, we have tested for the association of 6q25.1/rs6922269 and 9p21.3/rs1333049 with CHD in Pakistani population of Central Punjab. CHD case samples for this study were recruited from different hospitals in Gujranwala and Lahore Division, which are frequently visited by patients from all over Punjab. All-time smokers were found to be abundant in patients (53%) when compared to controls (32%). This finding highlights the significance of smoking as a risk factor for CHD as established by previous studies (Huxley and Woodward, 2011). Surprisingly, average BMI was lower in CHD cases (25.59 Kg/m^2) compared to controls (26.35 Kg/m^2). This could possibly be a result of careful eating, maintaining a healthy weight and exercise, as CHD patients are usually advised to change their lifestyle habits and become more active. Diabetes and hypertension were also more prevalent in cases as compared to controls which reinforces their recognition as risk factors for CHD (Erbel *et al*., 2012; Peters *et al*., 2014). In our cohort the frequency of the risk allele [A] for rs6922269 was higher in cases as compared to controls (0.20 vs 0.13; *P*=0.002). Previous studies also described a higher frequency of allele A in CHD cases (0.27 vs 0.26) of European origin (CADC, 2009). However, some other studies, where no association between rs6922269 [A] and CHD was found, gave opposite results. For example, lower A allele frequency was reported in CHD cases (0.22 vs 0.23) belonging to a Tunisian population (Ghazouani *et al*., 2010). Our study showed that the locus 6q25.1 was significantly associated with risk of CHD in population of Central Punjab (*P*=0.012). Additionally, the risk allele, A, was associated with an elevated risk of CHD in this population (OR=1.36, 95% CI=0.52-3.52). Previously, this SNP was reported to have an association with risk of CHD in combined analysis of German and UK populations ($P=2.9\times10^{-8}$) and an independent analysis of European populations (*P*=0.020) (Samani *et al*., 2007; CADC, 2009). It was also documented to have an association with incident CHD in American Whites ($P=5.1\times10^{-10}$) and to predict cardiovascular mortality (Franceschini *et al*., 2011; Hubacek *et al*., 2016). On the contrary, some other studies found no association between rs6922269 and risk of CHD or MI (Muendlein *et al*., 2009; Ghazouani *et al*., 2010). In our study, the observed significant association of rs1333049 with risk of CHD is mainly attributed to increased risk

allele frequency in cases compared to controls (0.55 vs 0.45; P=0.002). Previous reports have also shown a higher risk allele frequency in CHD or MI cases than in controls for other populations, including for example, 0.52 vs 0.49 in Japanese and 0.56 vs 0.46 in Han Chinese populations (Hiura *et al*., 2008; Peng *et al*., 2009). A study in Pakistani population also reported the association of C allele of rs1333049 with increased risk of MI (OR=1.55, 95% CI=1.22-1.96) (Ahmed *et al*., 2013). We also found out that the 9p21.3/rs1333049 was significantly associated with risk of CHD in Pakistani population (P=0.001). The risk allele, C, was observed to be associated with an increased risk of CHD in our population (OR=1.65; 95% CI=1.10-2.48). The association p value reported by the original study was very high (3×10^{-19}) and such strong signal has not been replicated in any of the later association studies (Samani *et al*., 2007). Later replication studies have revealed a strong association between rs1333049 and risk of CHD or MI in Japanese (OR=1.30; 95% CI=1.13-1.49; P=0.00027), European (OR=1.24; 95% CI=1.20-1.29; $P=6.04\times10^{-10}$) and East Asian (OR=1.29; 95% CI=1.23-1.36; P=0.001) populations, for instance (Hinohara *et al*., 2008; Schunkert *et al*., 2008; Guo *et al*., 2013). The PROMIS study in MI patients of Pakistan showed an odds ratio of 1.31 ($p=2\times10^{-3}$) per copy of the risk allele in subjects experiencing MI (Saleheen *et al*., 2010). The reported association of risk allele of rs1333049 with CHD in our population is, therefore, in accordance with the findings of previous studies. Our study is, however, limited because of the inclusion of a limited number of participants. Another limitation of this study is the non-random sampling of controls as they were selected based on convenience for CHD.

CONCLUSION

We have demonstrated association of rs6922269[A] and rs1333049[C] polymorphisms with CHD in a population of Central Punjab, Pakistan. There is, however, need to study these loci, especially rs6922269, in more detail in a larger sample to fully establish their role in the pathogenesis of CHD, as their exact functional relevance is yet to be known.

ACKNOWLEDGEMENTS

We acknowledge all the participants for donating their blood to conduct this study and the staff of various hospitals in Lahore, and Gujranwala for their help in sample collection.

Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2018.50.6.2295.2300

Statement of conflict of interest

We declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

Ahmed, W., Ali, I.S., Riaz, M., Younas, A., Sadeque, A., Niazi, A.K., Niazi, S.H., Ali, S.H.B., Azam, M. and Qamar, R., 2013. Association of ANRIL polymorphism (rs1333049: C> G) with myocardial infarction and its pharmacogenomic role in hypercholesterolemia. *Gene*, **515**: 416-420. https://doi.org/10.1016/j.gene.2012.12.044

Angelakopoulou, A., Shah, T., Sofat, R., Shah, S., Berry, D.J., Cooper, J., Palmen, J., Tzoulaki, I., Wong, A., Jefferis, B.J. and Maniatis, N., 2011. Comparative analysis of genome-wide association studies signals for lipids, diabetes, and coronary heart disease: Cardiovascular biomarker genetics collaboration. *Eur. Heart J.*, **33**: 393-407. https://doi.org/10.1093/eurheartj/ehr225

Cluett, C., McDermott, M.M., Guralnik, J., Ferrucci, L., Bandinelli, S., Miljkovic, I., Zmuda, J.M., Li, R., Tranah, G., Harris, T. and Rice, N., 2009. The 9p21 myocardial infarction risk allele increases risk of peripheral artery disease in older people. *Circ. Cardiovasc. Genet*., **2**: 347-353. https://doi.org/10.1161/CIRCGENETICS.108.825935

Coronary Artery Disease Consortium., 2009. Large scale association analysis of novel genetic loci for coronary artery disease. *Arterioscl. Thromb. Vasc*., **29**: 774-780.

Erbel, R., Lehmann, N., Möhlenkamp, S., Churzidse, S., Bauer, M., Kälsch, H., Schmermund, A., Moebus, S., Stang, A., Roggenbuck, U. and Bröcker-Preuß, M., 2012. Subclinical coronary atherosclerosis predicts cardiovascular risk in different stages of hypertension: Result of the Heinz Nixdorf Recall Study. *Hypertension*, **59**: 44-53. https://doi.org/10.1161/HYPERTENSIONAHA.112.193128

Franceschini, N., Carty, C., Bůžková, P., Reiner, A., Garrett, T., Lin, Y., Vöckler, J.S., Hindorff, L.A., Cole, S.A., Boerwinkle, E. and Lin,

D.Y., 2011. Association of genetic variants and incident coronary heart disease in multi-ethnic cohorts: The PAGE Study. *Circ. Cardiovasc. Genet*., **4**: 661-672. https://doi.org/10.1161/CIRCGENETICS.111.960096

Gaziano, T.A., Bitton, A., Anand, S., Abrahams-Gessel, S. and Murphy, A., 2010. Growing epidemic of coronary heart disease in low-and middle-income countries. *Curr. Probl. Cardiol*., **35**: 72-115. https://doi.org/10.1016/j.cpcardiol.2009.10.002

Ghazouani, L., Khalifa, S.B.H., Abboud, N., Perret, C., Nicaud, V., Khalfallah, A.B., Alamawi, W.Y., Cambien, F. and Mahjoub, T., 2010. Association of three polymorphisms selected from a genome-wide association study with coronary heart disease in the Tunisian population. *J. Thromb. Thrombolys*., **29**: 114. https://doi.org/10.1007/s11239-009-0336-0

Guo, J., Li, W., Wu, Z., Cheng, X., Wang, Y. and Chen, T., 2013. Association between 9p21.3 genomic markers and coronary artery disease in East Asians: a meta-analysis involving 9,813 cases and 10,710 controls. *Mol. Biol. Rep*., **40**: 337-343. https://doi.org/10.1007/s11033-012-2450-x

Hinohara, K., Nakajima, T., Takahashi, M., Hohda, S., Sasaoka, T., Nakahara, K.I., Chida, K., Sawabe, M., Arimura, T., Sato, A. and Lee, B.S., 2008. Replication of the association between a chromosome 9p21 polymorphism and coronary artery disease in Japanese and Korean populations. *J. Hum. Genet*., **53**: 357-359. https://doi.org/10.1007/s10038-008-0248-4

Hirschhorn, J.N., Lohmueller, K., Byrne, E. and Hirschhorn, K., 2002. A comprehensive review of genetic association studies. *Genet. Med.*, **4**: 45-61. https://doi.org/10.1097/00125817-200203000-00002

Hiura, Y., Fukushima, Y., Yuno, M., Sawamura, H., Kokubo, Y., Okamura, T., Tomoike, H., Goto, Y., Nonogi, H., Takahashi, R. and Iwai, N., 2008. Validation of the association of genetic variants on chromosome 9p21 and 1q41 with myocardial infarction in a Japanese population. *Circ. J*., **72**: 1213-1217. https://doi.org/10.1253/circj.72.1213

Hubacek, J., Stanek, V., Poledne, R., Adamkova, V. and Pitha, J., 2016. Rs6922269 marker at the MTHFD1L gene predict cardiovascular mortality in males after acute coronary syndrome. *Atherosclerosis*, **252**: e77-e78. https://doi.org/10.1016/j.atherosclerosis.2016.07.489

Huxley, R.R. and Woodward, M., 2011. Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. *Lancet*, **378**: 1297-1305. https://doi.org/10.1016/S0140-6736(11)60781-2

Johnson, D.C., Weinhold, N., Mitchell, J.S., Chen, B., Kaiser, M., Hillengass, J., Bertsch, U., Gregory, W.A., Cairns, D., Jackson, G.H. and Försti, A., 2016. Genome-wide association study identifies variation at 6q25. 1 associated with survival in multiple myeloma. *Nat. Commun*., **7**: 10290. https://doi.org/10.1038/ncomms10290

Karvanen, J., Silander, K., Kee, F., Tiret, L., Salomaa, V., Kuulasmaa, K., Wiklund, P.G., Virtamo, J., Saarela, O., Perret, C. and Perola, M., 2009. The impact of newly identified loci on coronary heart disease, stroke and total mortality in the MORGAM prospective cohorts. *Genet. Epidemiol*., **33**: 237-246. https://doi.org/10.1002/gepi.20374

McPherson, R., Pertsemlidis, A., Kavaslar, N., Stewart, A., Roberts, R., Cox, D.R., Hinds, D.A., Pennacchio, L.A., Tybjaerg-Hansen, A., Folsom, A.R. and Boerwinkle, E., 2007. A common allele on chromosome 9 associated with coronary heart disease. *Science*, **316**: 1488-1491. https://doi.org/10.1126/science.1142447

Mensah, G.A., Forouzanfar, M.H., Naghavi, M., Lozano, R., Ezzati, M., Moran, A., Feigin, V., Vos, T. and Murray, C.J., 2013. Comparable estimates of mortality and trends for cardiovascular diseases including congenital heart disease in 21 world regions in 1990 and 2010: The global burden of diseases, injuries and risk factors study. *J. Am. Coll. Cardiol*., **61**: e1406. https://doi.org/10.1016/S0735-1097(13)61406-0

Muendlein, A., Saely, C.H., Rhomberg, S., Sonderegger, G., Loacker, S., Rein, P., Beer, S., Vonbank, A., Winder, T. and Drexel, H., 2009. Evaluation of the association of genetic variants on the chromosomal loci 9p21.3, 6q25.1, and 2q36.3 with angiographically characterized coronary artery disease. *Atherosclerosis*, **205**: 174-180. https://doi.org/10.1016/j.atherosclerosis.2008.10.035

Pagliardini, L., Gentilini, D., Panina-Bordignon, P., Busacca, M., Candiani, M. and Di Blasio, A.M., 2013. An Italian association study and meta-analysis

with previous GWAS confirm WNT4, CDKN2BAS and FN1 as the first identified susceptibility loci for endometriosis. *J. Med. Genet*., **50**: 43-46. https://doi.org/10.1136/jmedgenet-2012-101257

Parle-McDermott, A., Pangilinan, F., O'brien, K.K., Mills, J.L., Magee, A.M., Troendle, J., Sutton, M., Scott, J.M., Kirke, P.N., Molloy, A.M. and Brody, L.C., 2009. A common variant in MTHFD1L is associated with neural tube defects and mRNA splicing efficiency. *Hum. Mutat*., **30**: 1650-1656. https://doi.org/10.1002/humu.21109

Peng, W.H., Lu, L., Zhang, Q., Zhang, R.Y., Wang, L.J., Yan, X.X., Chen, Q.J. and Shen, W.F., 2009. Chromosome 9p21 polymorphism is associated with myocardial infarction but not with clinical outcome in Han Chinese. *Clin. Chem. Lab Med*., **47**: 917-922. https://doi.org/10.1515/CCLM.2009.215

Peters, S.A., Huxley, R.R. and Woodward, M., 2014. Diabetes as risk factor for incident coronary heart disease in women compared with men: a systematic review and meta-analysis of 64 cohorts including 858,507 individuals and 28,203 coronary events. *Diabetologia,* **57**: 1542-1551. https://doi.org/10.1007/s00125-014-3260-6

Ren, R.J., Wang, L.L., Fang, R., Liu, L.H., Wang, Y., Tang, H.D., Deng, Y.L., Xu, W., Wang, G. and Chen, S.D., 2011. The MTHFD1L gene rs11754661 marker is associated with susceptibility to Alzheimer's disease in the Chinese Han population. *J. Neurol. Sci*., **308**: 32-34. https://doi.org/10.1016/j.jns.2011.06.036

Saade, S., Cazier, J.B., Ghassibe-Sabbagh, M., Youhanna, S., Badro, D.A., Kamatani, Y., Hager, J., Yeretzian, J.S., El-Khazen, G., Haber, M. and Salloum, A.K., 2011. Large scale association analysis identifies three susceptibility loci for coronary artery disease. *PloS One*, **6**: e29427. https://doi.org/10.1371/journal.pone.0029427

Saleheen, D., Alexander, M., Rasheed, A., Wormser, D., Soranzo, N., Hammond, N., Butterworth, A., Zaidi, M., Haycock, P., Bumpstead, S. and Potter, S., 2010. Association of the 9p21. 3 locus with risk of first-ever myocardial infarction in Pakistanis: case-control study in South Asia and updated meta-analysis of Europeans. *Arterioscl. Thromb. Vasc*., **30**: 1467-1473. https://doi.org/10.1161/ATVBAHA.109.197210

Samani, N.J., Erdmann, J., Hall, A.S., Hengstenberg, C., Mangino, M., Mayer, B., Dixon, R.J., Meitinger, T., Braund, P., Wichmann, H.E. and Barrett, J.H., 2007. Genomewide association analysis of coronary artery disease. *New Engl. J. Med*., **357**: 443-453. https://doi.org/10.1056/NEJMoa072366

Saqlain, M., Kalsoom, H., Fiaz, M., Mahmood, A., Qazi, R.A., Safdar, W., Shaiq, P.A., Cheung, B.M. and Raja, G.K., 2018. Association of intestinal fatty acid binding protein (FABP2) polymorphism with metabolic syndrome risk. *Pakistan J. Zool.,* **50**: 175-181. http://dx.doi.org/10.17582/journal.pjz/2018.50.1.175.181

Schunkert, H., Götz, A., Braund, P., McGinnis, R., Tregouet, D.A., Mangino, M., Linsel-Nitschke, P., Cambien, F., Hengstenberg, C., Stark, K. and Blankenberg, S., 2008. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21. *Circulation*, **117**: 1675-1684. https://doi.org/10.1161/CIRCULATIONAHA.107.730614

The Wellcome Trust Case Control Consortium, 2007. Associations between apolipoprotein B, apolipoprotein AI, the apolipoprotein B/AI ratio and coronary heart disease: a literature-based meta-analysis of prospective studies. *Nature*, 447: 661-678. https://doi.org/10.1038/nature05911

Thompson, A. and Danesh, J., 2006. *J. Intern. Med*., **259**: 481-492. https://doi.org/10.1111/j.1365-2796.2006.01644.x

Wei, Y., Xiong, J., Zuo, S., Chen, F., Chen, D., Wu, T., Guo, W. and Hu, Y., 2014. Association of polymorphisms on chromosome 9p21. 3 region with increased susceptibility of abdominal aortic aneurysm in a Chinese Han population. *J. Vasc. Surgery*, **59**: 879-885. https://doi.org/10.1016/j.jvs.2013.10.095

Yu, J.T., Yu, Y., Zhang, W., Wu, Z.C., Li, Y., Zhang, N. and Tan, L., 2010. Single nucleotide polymorphism rs1333049 on chromosome 9p21. 3 is associated with Alzheimer's disease in Han Chinese. *Clin. Chim. Acta*, **411**: 1204-1207. https://doi.org/10.1016/j.cca.2010.06.013

Zheng, C., Yang, H., Wang, Q., Rao, H. and Diao, Y., 2016. Association analysis of five SNP variants with gout in the Minnan population in China. *Turk. J. med. Sci*., **46**: 361-367. https://doi.org/10.3906/sag-1409-58