REVIEW ARTICLE

A SYSTEMATIC REVIEW OF GENETIC FACTORS INVOLVED IN CARDIOVASCULAR DISORDERS

Supervisor: Dr. Shumaila Zulfiqar

Submitted by: Azka Abid

Registration No: F18BGEN001

Kinnaird College for Women University, Lahore

Department of biotechnology

Session: 2018-2022

RESEARCH COMPLETION CERTIFICATE

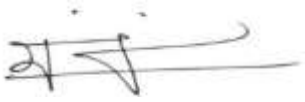
It is certified that Ms. Azka Abid of BSc- Hons of session 2018-2022 (registration No. F18BGEN001), Department of Biotechnology has carried out research work entitled **"Genetic Elucidation of Cardiovascular Disorders".**

"All changes suggested by examiners during defense are incorporated in this final copy"

Research Supervisor

Dr. Shumaila Zulfiqar
Assistant Professor
Department of Biotechnology
Kinnaird College for Women

Head Of Department



Dr. Iram Anjum
Associate Professor
Department of Biotechnology
Kinnaird College for Women

Dated: June 16, 2022

ANTI-PLAGIARISM
DECLARATION

I Azka Abid, hereby certify that this is my own research work. The work has not, in whole or in part, been presented elsewhere for assessment. Where material has been used from other sources,it has been properly acknowledged. The similarity index of the research report is **9%**. If this statement is untrue and I am found guilty of plagiarism, the punitive actions against me should be taken as per Kinnaird Anti-Plagiarism Policy.

Azka Abid
F18BGEN001
BSc(Hons) Biotechnology Signature:

Research Supervisor

Dr. Shumaila Zulfiqar
Assistant Professor Department of Biotechnology
Kinnaird College for Women

DECLARATION

I, Azka Abid, hereby declare that the material printed in the thesis is my original work and has been carried out under the supervision of Dr. Shumaila Zulfiqar. To the best of my knowledge, this research work does not contain any material that has been submitted for obtaining similar degree from any other educational institute.

Azka Abid

Dedicated

To

My Beloved Father, Abid Ali

&

My dearest Mother, Qurat-ul-Ain Ali

ACKNOWLEDGEMENTS

All praise to be Allah Almighty, Lord of the worlds. I am grateful beyond words for His countless blessings on me and my loved ones. This project would not have been possible without support of many people. I wish to express my heartiest thanks and gratitude to my parents for always believing in me and helping me in everything I wanted to accomplish. Their care and love for me is beyond explanation. A profound thanks to Dr. Shahnaz Choudhry, Academic Dean and Dr. Irum Anjum Head of Biotechnology Department, for providing me an excellent research facilities.

I would like to extend my gratitude to all my instructors throughout my degree who have taught and guided me; it is their hard that has brought me to the conclusion of my degree. First and foremost, I would like to thank my supervisor Dr. Shumaila Zulfiqar. She has been an ideal mentor, supervisor, encouraging with a perfect blend of intuition. I am grateful for my time working with her. I am indebted to her for providing me her extraordinary experience, unflinching support, and encouragement. I would like to convey my special gratitude to all my teachers in the Biotechnology Department for enlightening me about the courses they have taught me and instilling a general love for Biotechnology in me through their deep knowledge and expertise. I express my cordial thanks and sincerest gratitude to my family for always having my back. My journey of undergraduate research has been a thoroughly interesting one. All of this is the outcome of untiring effort and good wishes moral and financial support of my loving parents so I owe my deepest regards to them.

Table of Contents

Table of Figures:

Abstract

Background:

Cardiovascular disorder is the leading causality of death around the world. Cardiovascular disorders have multiple environmental and genetic risk factors contributing towards different types of CVD. This study aimed to investigate most prevalent type of CVD among Pakistani population.

Methods:

Total 50 clinical reports were collected from patients by visiting different private and public health institutes in Lahore, Pakistan. The thorough literature review was performed by using different databases including Google scholar and PubMed to analyze different types of cardiovascular diseases. SPSS software version 16.0 was used to analyze the results.

Results:

Our results showed that Rheumatic heart disease is the most usual type of heart disease in Pakistan (34.0%) whereas; Left ventricular systolic function (LVF) and Patent ductus arteriosus (PDA) are the least reported cases (2.0%)

Conclusion:

Our findings suggests that broad clinical and genetic spectrum of cardiovascular disorders in Pakistan.

Keywords

Genetic elucidation, Cardiovascular disorders, Epigenetics, Mutation, Novel variants

Introduction:

The unique characteristics and instructions for the functioning of cells are all contained in DNA (deoxyribonucleic acid) which forms segments called genes. Harmful changes in the genes (mutations) occur due to mutagens or inheriting a mutated gene from parents hence leading to the development of genetic disorders (1). The etiology for many disorders has been discovered. OMIM (Online Inheritence in Man) lists 4339 gene variants affecting phenotype. Orphanet lists more than 7800 relationships between disease and gene (2).Cardiovascular disorders (CVDs) is the leading cause of death around the world. The class of diseases described by CVD affects heart and blood vessels such as coronary artery disease, cardiomyopathies, arrhythmias or heart failure (3). The group of CVD includes coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease and deep vein thrombosis and pulmonary embolism (4). Obesity, smoking, high cholesterol or hypertension are included in number of risk factors that contribute to CVD. However, now it is known that these environmental risk factors contribute to a fraction of CVD cases. Therefore, the focus of researchers has been on genetic elucidation of cardiovascular disorder so the mechanisms that are independent of environmental risk factors can be identified (3). Long thought to be heritable, recent developments have begun to untangle the disease's genetic architecture. About 60 genetic loci have been associated to coronary risk in common variant association studies. Large-scale gene sequencing initiatives and functional investigations have helped researchers better identify causal risk factors, elucidate underlying biology, and inform the creation of new therapies (5).

Clinical symptoms of cardiovascular disorders vary according to the type of CVD the patient has and they also vary from men to women. A buildup of fatty plaques in arteries can damage the blood vessels which can lead to pain, pressure, tightness and discomfort in chest. Men are more feasible to have chest pain as compared to women. There can be shortness of breath, numbness and pain in legs or arms in case of narrowed blood vessels in parts of the body. There can be neck pain, thorat, jaw or

Gene(s)	CVD phenotype	Inheritance pattern	Other related diseases
GDF1, DTGA3, DORV, RAI, CHTD6	Right atrial isomerism	Autosomal recessive	• Abdomen Situs inversus • Abdomen Situs ambiguous • Midline liver
ZIC3, HTX1, HTX, VACTERLX	Congenital heart defects, nonsyndromic, 1, X-linked	X-linked recessive	• Failure to thrive • Hypertelorism Dextrocardia • Lung lobation defects • Bilobed right lung
LDLRAP1, ARH, FHCB2, FHCB1, FHCL4	Familial hypercholesterolemia, 4	Autosomal recessive	• Xanthomas • Hypertriglyceridemia
MYH7, TNNT2, TPM1, MYL3, MYL2, PLN, MYBPC3	Hypertrophic cardiomyopathy	Autosomal Dominant	• chest pain shortness of breath (palpitations) l • dizziness • fainting.
DMD, MYH7, TNNT2, MYH6, MYPN, MYBPC3, ANKRD1, RAF1, DES	Familial dilated cardiomyopathy	Autosomal dominant	• Normal neurologic examination • Adams-Stokes attacks
CTA2, MYH11, BN1, TGFBR1/2,	Familial Thoracic d dissection	Autosomal dominant	• mild features of Marfan syndrome

OL3A1, LOX , GFB2/3			• sudden chest or back pain • pallor • paresthesias
KCNQ1/H2/J2/E1, SCN5A,CALM1/2, CAV3,	Long QT syndrome	Autosomal dominant	• Unexplained fainting, drownings, seizures or other accident
SCN5A	Brugada syndrome	Autosomal dominant	• Right bundle branch block and ST segment elevation on ECG • Idiopathic ventricular fibrillation • Cardiac arrest • Sudden death
KCNH2	Short QT syndrome	Autosomal Dominant	• Andersen-Tawil syndrome (ATS) • long QT syndrome type 2
ABCG5, ABCG8	Sitosterolemia	Autosomal recessive	• Xanthelasma • Arcus corneae • Splenomegaly • Arthralgia • Arthritis
FBN1, TGFBR2, SMAD3, TGFBR1,	Marfan's syndrome	Autosomal dominant	• Emphysema in most severe presentation • Pneumothorax

TGFB2, SKI, TGFB3			• Pulmonary blebs • Scoliosis • Kyphoscoliosis • Thoracic lordosis • Spondylolisthesis
BMPR2, CAV1, KCNK3, BMPR1B, SMAD9, ENG, ACVRL1, EIF2AK4	Pulmonary arterial hypertension	Autosomal dominant	• Dyspnea • Arterial hypoxemia
TAPVR1	Anomalous pulmonary venous return	Autosomal dominant	• Hypoplastic right Lung Frequent respiratory infections

Table: This shows the genes related to different types of Cardiovascular disorders and their association with other diseases. The genes are searched from OMIM.

back pain. Coronary artery disease can be diagnosed once you have angina, stroke or failure of heart. Heart arrhythmias show the clinical symptoms of having slow heartbeat, dizziness, fainting, tachycardia or fluttering in chest. Serious congenital heart defects show the symptoms of cyanosis, swelling in abdomen, legs or around eyes in baby. Less serious congenital heart defects can show the symptoms of short breath during an activity, swelling in hands or feet. Cardiomyopathy may show irregular heartbeats that feel fluttering. Heart infection can lead to fever, dry or constant cough and rashes on skin or unusual spots. Damage in heart valves can cause irregular heartbeat, fatigue, shortness of breath and syncope (6).

Asia has been shown to have a larger burden of CVD than western populations, with the majority of this burden being carried by economically disadvantaged communities, primarily in the region of South Asia. Pakistan is one of these underdeveloped countries that are just as afflicted by the cardiovascular pandemic as the rest of the world, yet there is relatively little registered data on the issue. A population study in Pakistan for prevalence of CVD was conducted which demonstrated that there is increased tendency of risk factors of CVD at younger age with prevalence in females (7).

The focus of this review is to target the genetic elucidation of multifactorial cardiovascular disorder and to present a comparative analysis in relation to Pakistan. A cross-sectional study conducted on prevalence of CVD in Punjab showed that 17.5% of population had cardiovascular disease which included 18.3% in females and 16.6% in males. The disease risk factors in study area included low level of activity, inactive lifestyle and family history. Thus, concluding that CVD is a serious problem for Pakistan affecting both genders (8)

Genome-wide association studies:

The identification of inherited conditions at an early stage has been enabled by identifying the polymorphism within individuals. Polymorphism in CDH13, MTHFD1L, CXCL12, OR13G1, CDKN2B, MRAS, SMAD3, MIA3, APC, LPL genes have been shown to influence the risk of CAD (coronary artery disease) through studies of genome-wide association and large meta analyses. This has helped a lot in today's world where people are moving towards personalized healthcare. In a recent study, genotyping has been used to identify the susceptibility of an individual towards heritable conditions and to help encounter genetic risk factors to adopt a healthy lifestyle and incorporate plans for prevention of disease (9).

Whole exome sequencing has also been used to identify nuclear envelope gene variants in patients with cardiovascular disorders in which heterozygous splice-site or non-sense variants were identified in nucleoporin genes: NUP188, NUP43 and NUP 37. Splice-site variant was also identified in nuclear envelop gene: SYNE1. Nucleoporin genes were identified as novel genes (10).

Genetic risk variants for abdominal aortic aneurysm have been identified by genome wide-association studies (GWAS). Eight case-control studies were included to recognize 33 SNPs associated with abdominal aortic aneurysmreporting CDKN2B antisense RNA1 gene having the most significant association (11). Meta-analysis and Mendelian randomization of clinical trials was conducted to prove the relationship between cardiovascular disorders, urate and blood pressure. The analysis of Mendelian randomization showed that coronary heart disease (odds ratio, 1.19 [95% CI, 1.10-1.30]; $P=4\times10^{-5}$), stroke (1.11 [95% CI, 1.05-1.18]; $P=2\times10^{-4}$) and peripheral artery disease (1.12 [95% CI, 1.03-1.21]; $P=9\times10^{-3}$) increased with 1-SD increase in serum urate. Meta-analysis showed that urate has a favorable effect on lowering the blood pressure. The clinical trial data concluded that blood pressure increases with higher serum urate which may effect on cardiovascular disease risk (12). Similarly, the association between cardiovascular disorders with high serum uric acid level was determined using Mendelian randomization approaches in which 28 genetic variants linked to uric acid were used hence concluding that study did not

supported clinically causal effect of serum urate determined genetically on cardiovascular disorders because of pleiotropic effects (13). It suggests that there is a further need of uric acid genes study to elucidate the association between cardiovascular disorders and uric acid levels (14).

Epigenetics:

Environmental factors cause epigenetic modifications which affect the expression of genes in an individual in process of disease development. The aspects of epigenetics during in-utero exposure and after birth have not been explored adequately (15). Another research showed that the deficiency of breast cancer genes 1 and 2 (BRCA1/2) is implicated in progression of cardiovascular disease as they mediate the activity of Nrf2 which is a potential mediator of cardiovascular disorders caused due to metabolic diseases (16).

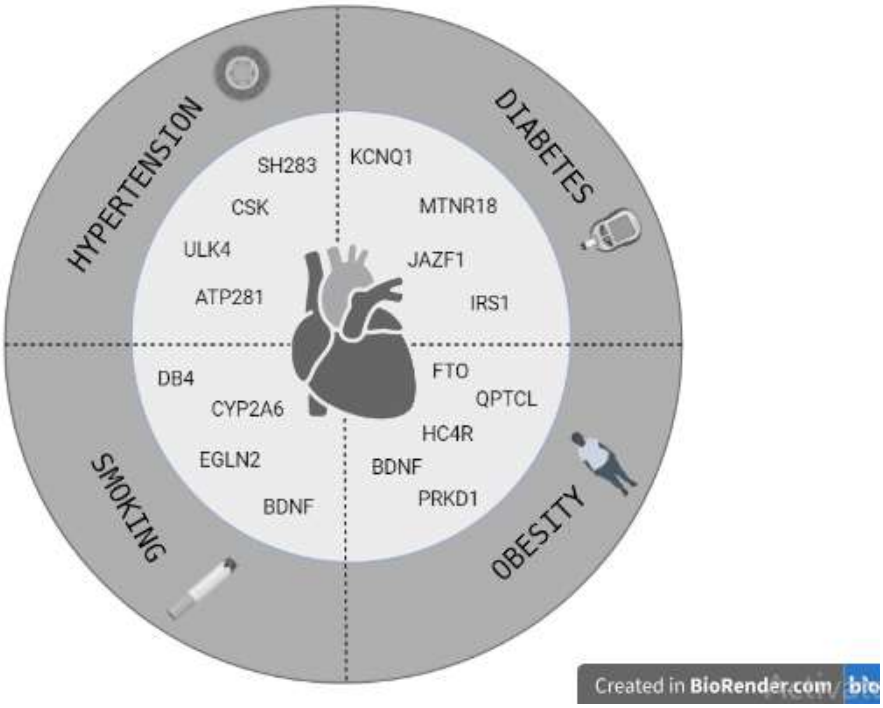


Figure 1: Four non-genetic risk factors for CVD under genetic control. Some of the selected CVD genes associated with subsequent risk factors have been shown.

Systematic reviews have been evaluated to show the association between cardiovascular outcomes and adiposity. Mendelian randomization and observational studies were assessed to gather epidemiological relationships showing that adiposity is one of the causality for CVD (17). Animal models and analysis of mutations were overviewed in recent researches to understand the molecular pathways and mechanism in a better way and linking it to cardiovascular development and CHD. It identified that 22q11.2 deletion syndrome is associated highly with CHD. The comprehensive expression analysis and usage of pluripotent stem cells procedures elucidated spatial and temporal gene regulatory mechanism using next generation sequencing (18).

Mutations leading to the development of cardiovascular disorder:

Cardiovascular disorders are multifactorial disorders and more than 50 gene mutations have been associated with dilated cardiomyopathy (CDM) development. CRISPR/Cas9 was used to edit nucleotides in zebrafish to model cardiovascular disorders in humans. 4 different knock-in cell lines were generated carrying missense mutation genes causing CVD in humans and were combined in orthologous genes of zebra fish through CRISPR/Cas9. Gain of function mutation in genes Kir6.1 and KCNJ8 encoding pore formation and regulatory genes ABCC9 and SUR2, displayed enlarged ventricles hence demonstrating causality of Cantú syndrome. The results lead to the ways of new therapeutic drugs through broad application for human genetic disease modeling (19). Another study demonstrated the role of TRIM37 in MULIBREY genetic disorders in relevance to cardiovascular disorders. MULIBREY syndrome constitute of myocardial fibrosis, pericarditis, hypertrophy as most common heart manifestations. Lipsanen-Nyman et al. demonstrated a study of 49 male patients of MULIBREY syndrome and followed them for 25 years showing that half of them had congestive heart failure, 20 % had died due to cardiac arrest. Autopsies showed the presence of myocardial fibrosis and myocardial hypertrophy. TRIM37 deficiency in children also correlated negatively with the size of left ventricle (20).

There are still many unidentified risk factors of CVD. The accumulation of DNA mutations in somatic cells is one of the major players in CVD. This study reported the association between somatic mutations and CVD in any of 160 genes where mutations in just four genes (TET2, DNMT3A, JAK2 and ASXL1) account for more than 70% of mutations detected. In this study more than 5000 cancer free blood cells where analyzed through whole-exome sequencing hence reporting the development of atherosclerotic CVD increase with more than 2-fold due to mutations in hematopoiesis-related genes (21). Differences in mitochondrial biology may be partially related to sex-specific cardiac functions. Mitochondrial mutations have more deleterious effects in males because natural selection operates only in female mitochondria. Thus the mitochondrial deleterious mutations are responsible for large panel of disorders including cardiomyopathy and renal dysfunctions (22).

Nuclear proteins are encoded by Lamin A and Lamin C isoforms and mutations in LMNA can result in conduction disorders and cardiomyopathy. A study employed iPSCs (induced pluripotent stem cells) from human cells carrying heterozygous mutation K219T on LMNA to create a disease model and as a result cardiomyocytes differentiated form these induced pluripotent stem cells. SCN5A loss of functional mutations leads to the defective production of $Na_v1.5$ proteins which causes minor changes in structure of heart. Correction of these mutations mediated by CRISPR/Cas-9 reestablishes SCN5A epigenetic expression and sodium current density which is responsible for normal cardiac electricity, thus polycomb repressive complex 2 cooperates with K219T-LMNA leading to slower velocity of conduction. This mechanism underlies the conduction abnormalities related with cardiac laminopathy (23, 24). A research study showed that mutation in α- galactosidase A affects the longitudinal function of left ventricle leading to cardiac disorders. This research included twenty-four heterozygous females with mutation of α-galactosidase and without hypertrophy of left ventricle, which underwent magnetic resonance using ^{18}F-FDG and cardiac positron emission topography along with echocardiography. The results showed that early sign of myocardial damage was represented by α-galactosidase A mutation (25). Congenital disorders were led by mutations in NOTCH signaling pathway genes through a wide range of spectrum. Four NOTCH

mutations were studied from which 3 showed the association with cardiovascular disorders. NOTCH 1 mutations lead to the left-side congenital heart disease, NOTCH 2 mutation lead to Alagille syndrome development and NOTCH 3 mutation causes arteriopathy with subcortical infarcts. However NOTCH 4 mutations have not been associated with cardiovascular disorders (26).

Cardiovascular risk factors in Pakistan:

In Pakistan about 46% of deaths occur due non-communicable disorders linked with obesity from which 455 deaths occur due to CVD. The rate of CVD mortality in Pakistan has shown to be increased in individuals that have central fat deposition and are overweight (27). A case control study done in Pakistan including a total of 970 individuals, to find out the role of FTO gene in CAD and obesity, showed the significant association of FTO gene polymorphism rs9939609 with both CAD (coronary artery disease) and obesity among Pakistani population (28). In Pakistan every year 700 children with genetic disorders are born due to consanguineous marriages. Children born with hereditary heart disorders are 2.3 times more probable to be born of cousin marriages as compared to children born without hereditary heart disorders (29). A research done on the prevalence of cardiovascular disorders in Punjab, Pakistan included 6351 individuals from which 17.5% were cardiovascular patients. The research also showed that prevalence is less in males (16.60%) than in females (18.30%) and females were more prone to the CVD at an early age than males (8).

Pakistan was graded as 2nd largest country for providing shelter to refugee (1.6 million). Pakistan is currently fronting the problem of internally displaced people (IDPs) due to economic and political instability, terrorism and natural disasters. In a study on health problems in refugees in Pakistan the data was collected from regions where there is larger influx of refugees. The rate of cardiovascular disorders is higher among refugees due to starvation and stress which might occur due to force migrations. The afghan refugees has cardiac rate of 6.67/1000 in patients in Pakistan. 20 cases in 3 years, of cardiovascular disorders were reported among IDPs in Peshawar district and in 2 years 2,685 cases were reported form Bannu district (30).

Toxic metal pollution has increased many folds in Pakistan and it is having serious health problems to the public. The intake of Pb, Ni, Cd, Cr and Cu causes problems in heart whereas the Mg can lead to hypertension. Studies have shown that overexposure in air of these heavy metals can result in cardiovascular disorders (31). In Pakistan 34% of the cities showed mean level of fluoride higher than 1.5mg/L among which Quetta, Lahore and Tehsil Mailsi had maximum values of 24.48, 23.60 and more than 5.5mg/L. Extended exposure to higher levels of fluoride through drinking water have resulted in higher rate of cardiovascular disorders in Pakistan (32). Massive influx of traffic and rapid urbanization has deteriorated the health status of Islamabad. The long exposure to principally particulate matter (PM) 2.5 is attributable to health endpoint causing 26 deaths for heart problems whereas short-term exposures attributed to 38 admissions in hospitals in Islamabad for cardiovascular disorders (33).

Usual risk factors of coronary artery disease like gender, hypertension, age, smoking provide modest discrimination however the analysis of GRS (genetic risk score) may provide risk factors above conventional risk factors. A study was conducted to analyse coronary artery disease genetic risk in Pakistan using score of genetic risk. Genetic risk score of SNPs at 21 loci in 18 genes form Pakistani population were analyzed to examine their combined effect on coronary artery disease (CAD). The results showed that one single nucleotide polymorphisms were not associated with coronary artery disease except FTO and APOB. The genetic risk score was quantitatively linked with risk of CAD and showed association with lipid level of blood (34). GRS was constructed form polymorphism in 4 genes (IL-6, PON1, ALDH2 and ITGB3T) associated with coronary heart disease in Pakistani patients. Serum lipid profiles were measured and statistical analysis was performed using SPSS 22.0, the GRS was also calculated from individual single nucleotide polymorphism and the association between them was examined using logistic regression. Genetic risk score represented significant association with coronary artery disease when none of the single variants showed statistically significant connection with CAD (35). To reveal in-depth association of CAD risk factors with individual lipids a novel approach of Direct infusion high-resolution mass spectrometry (DIHRMS) was performed on 5662 healthy participants from Pakistan. The DIHRMS

method provided 11.6% variation of median coefficient exhibiting high association of lipids with clinical chemistry biomarkers hence providing a novel insight into the genetic relationship of lipids and risk factors of coronary heart disease in Pakistan (36).

A SNP rs10911021 present upstream of GLUL gene was shown to be associate with CHD in T2DM (Type 2 diabetes mellitus) patients. The association of this SNP with coronary heart disease and oxidative stress biomarkers was checked in Pakistani patients through genotyping. It was shown that rs10911021 SNP was linked with coronary heart disease only in patients having diabetes and showed significant association with oxidative stress in CHD (coronary heart disease) hence concluding that this SNP contribute to increase the heart disease risk in diabetics by increasing oxidative stress (37).

Novel variants reported in Pakistan:

The first report of mutation in TNN13K gene in population of Pakistan was outlined in 2021. A consanguineous family in Pakistan with four patients of cardiac conduction disease was involved in the study. Co-segregation analysis and Whole exome sequencing analysis was performed to determine novel missense mutation in TNN13K encoding genes. The mutant variants changes the structural function of troponin I-interacting kinase hence leading to disease state (38). Due to rapid expansion of Pakistani population in recent times the genes affecting cardiovascular disorders have attained subtle deleterious SNVs. These deleterious variants were curated manually which had been reported already to be related with Mendelian, common and inherited CVDs. The greater proportion of filtered variants in Pakistani and south Asian populations were reported to be 8.8% associated with cardiac arrhythmia, 23.9% with long QT_syndrome, 47.8% with cardiomyopathies and 5.0% with ventricular septal defects (39). The prioritized variants in south Asians is persistent due to higher consanguinity where cardiomyopathies are associated with 25 base pair deletion in MYBPC3 with frequency of 4.0% in population. These prioritized variants can be used in new drug response strategies specific to this region (40).

Rheumatic heart disease is highly common in both rural (5.7 / 1000 individuals) and urban (22 / 1000 individuals) district of Pakistan. MEFV and TNF genes contribute to a very small RHD patients in Pakistan however a novel mutation (g.G2,096A) in MEFV gene exon 2 has also been reported (41). Hypertrophy cardiomyopathy gene variants of MT-RNR2, MT-TL1 and MT-TI were analyzed in Pakistani population. MT-RNR2 gene mutations mt-1811 A>G and mt-1888 A>G were observed for the first time in HCM patients of Pakistani population. Pathogenic mutations associated with hypertrophy cardiomyopathy were detected in mt-DNA encoded gene 16S-rRNA (42). Sinoatrial node is one the various structures for the cardiac conduction system and it regulates the rate of heart. From 5 Pakistani families 1 family showed a new missense variant in CACNA1D in p. (A376V), of sinoatrial dysfunction and deafness syndrome has been reported. 4 other family pedigrees defined the founder variant which is p.(G403-V404insG) (43).

The third most common cardiovascular disease is venous thromboembolism and is associated to the clot formation in vein. Prothrombin gene mutation in was reported to be 2.7%. Majority of the cases of the VTE (venous thromboembolism) study showed that patient with inherited thrombophilia case history had prothrombin gene mutation (44).

Methodology:

In this study, total 50 reports from cardiovascular patients were collected to analyze the rate of different types of cardiovascular disorders in Pakistani population and to present the most common type present in population. The reports that were collected from patients belonged to two different health institutes, Punjab Institute of Cardiology and Mayo Institute of Cardiology. The mean age of the patients included in the study 45.34 $\pm$ 14.66. SPSS (16.0) software was used to build the chart for different types

We also reviewed papers from 2012-2022.The articles related to genetic disorders were pertained from Google scholar and PubMed search engine on NCBI. The articles targeted for review focused on genetic elucidation of cardiovascular disorders.

The genes related to CVD and their associations with other disorders were searched from OMIM (Online Mendelian Inheritance in Man). The focus of the review studies were on genetic mechanisms of cardiovascular disorders and its prevalence in Pakistan. Further study of comparative analysis of genetic elucidations of cardiovascular disorders and role of environmental factors in CVD helped uncover genetic mechanisms underlying CVD and effect of environmental factors on epigenetics hence leading to Cardiovascular disorders. The available documented data on CVD in Pakistan were analyzed critically, that is a serious problem for the country affecting both the genders. This is the target problem around which our suggested prospect is designed. An overview of different types of cardiovascular disorders due to genetic mutations is given specifically existing in Pakistan. This is related to environmental factors in order to present a definite contrast in argument. The main purpose of this review is to establish a valid argument that helps in understanding genetic elucidations of cardiovascular disorder. Genetic explanation of cardiovascular disorders is needed to be explored and evaluated on the basis of its usual occurrence. The review has focused on genetic basis of cardiovascular disorders having high rate in Pakistan.

In total, **50** reports from cardiovascular patients were collected to analyze the prevalence rate of different types of cardiovascular disorders in Pakistani population and to present the most common type present in population. The reports that were collected from patients belonged to two different health institutes, Punjab Institute of Cardiology and Mayo Institute of Cardiology. The mean age of the patients included in the study 45.34 $\pm$ 14.66. SPSS software was used to build the chart for different types of cardiovascular disorders in Pakistan. BioRender software was used to create figure.

This review will exclude topics covering detailed association of cardiovascular disorders with other diseases as they have separate undefined set of problems that are not addressed.

Inclusion:

The study aims to target risk factors of cardiovascular disorders in Pakistan. Cases are also included to find the latest reported gene mutations associated with cardiovascular disorders. Mendelian randomization and observational studies are mentioned that were assessed to gather epidemiological relationships and insights between cardiovascular disorders and other environmental factors. Both the genders are included as according to studies in Pakistan and around the world it has stated that CVD has similar effect on both the genders. The environmental causes will also be addressed by linking it to genetics and epigenetics. The articles from last 10 years were included in the review. The clinical reports of Transthoracic Imaging were collected from cardiac patients of different health institutes of Lahore, Pakistan.

Exclusions:

The literature search will exclude the topics related to detailed association of cardiovascular disorders with other diseases. The articles that were published before 2012 were also excluded. Sporadic cases of cardiovascular disorders will also be excluded from the review article because among sporadic cases the risk of genes is often unknown. The clinical reports collected other than transthoracic imaging such as of angiography or echocardiogram were excluded.

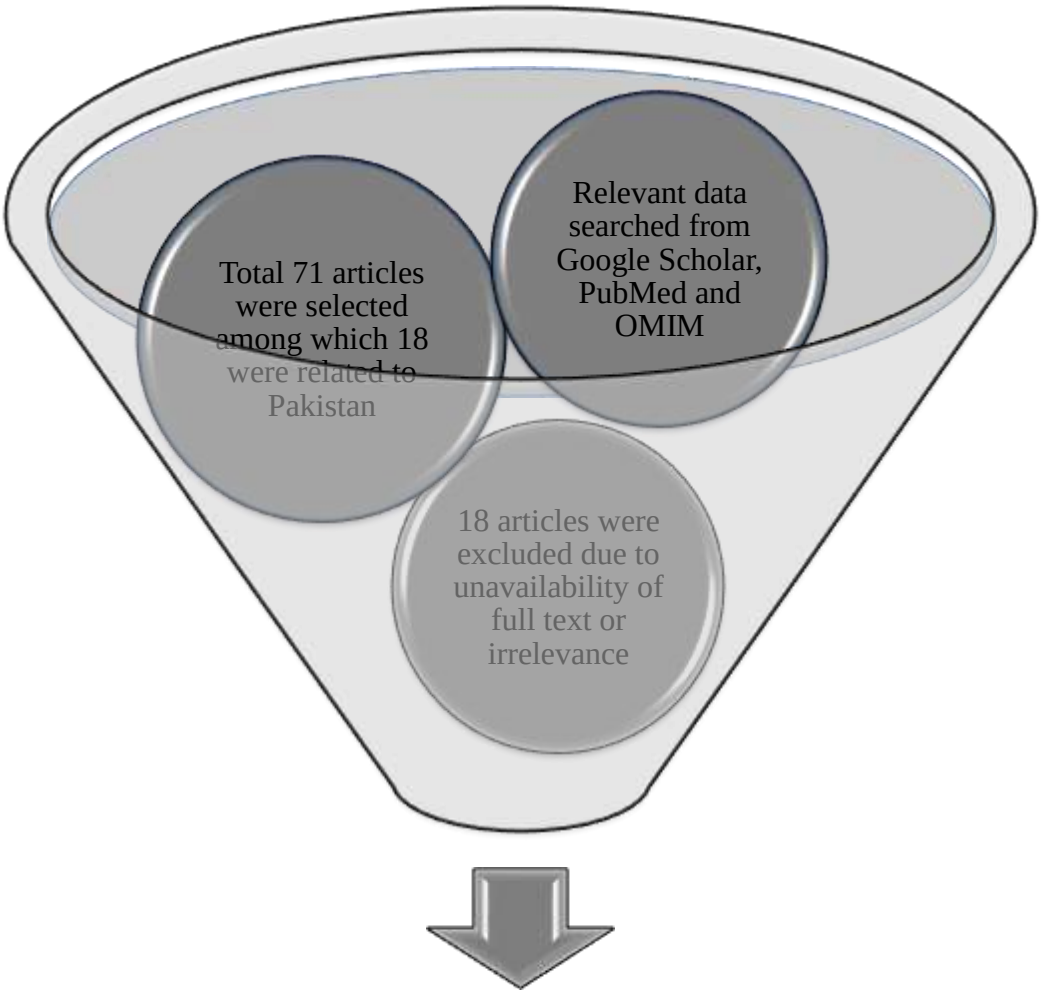


Figure 2: Flow Diagram showing the methodology of selecting articles

Results:

Statistics

heart_disease

N	Valid	50

Statistics

heart_disease

N	Valid	50
	Missing	0

Heart_disease

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	AS	2	4.0	4.0	4.0
	ASD	4	8.0	8.0	12.0
	BAV	2	4.0	4.0	16.0
	COA	2	4.0	4.0	20.0
	IHD	5	10.0	10.0	30.0
	large PDA	1	2.0	2.0	32.0
	LMCA	2	4.0	4.0	36.0
	LVH	2	4.0	4.0	40.0
	preserved LVF	1	2.0	2.0	42.0
	RHD	17	34.0	34.0	76.0
	small LAA	1	2.0	2.0	78.0
	SWMA	11	22.0	22.0	100.0
	Total	50	100.0	100.0	

The table shows the numbers of occurrences of heart disease are specified in the frequency column. Percentage column is where the number of occurrences of heart disease are divided by the total (50) multiplied by 100. This column shows that Rheumatic heart disease (RHD) has highest percentage of 34.0% whereas small left atrial appendage (LAA) and large patent ductus arteriosus (PDA)has lowest percentage of 2.0%. The other heart diseases including aortic stenosis (AS), bicuspid aortic valve (BAV), coarctation of aorta (COA), stenosis of left main coronary artery and left ventricular hypertrophy has 4.0% , atrial septal defect (ASD) has 8.0%, Ischemic heart disease (IHD) has 10.0% and segmental wall motion abnormalities has

22.0% prevalence in reported cases. The cumulative percent column is the total you get when you add the value of percentage column to each other as you descend towards the rows.

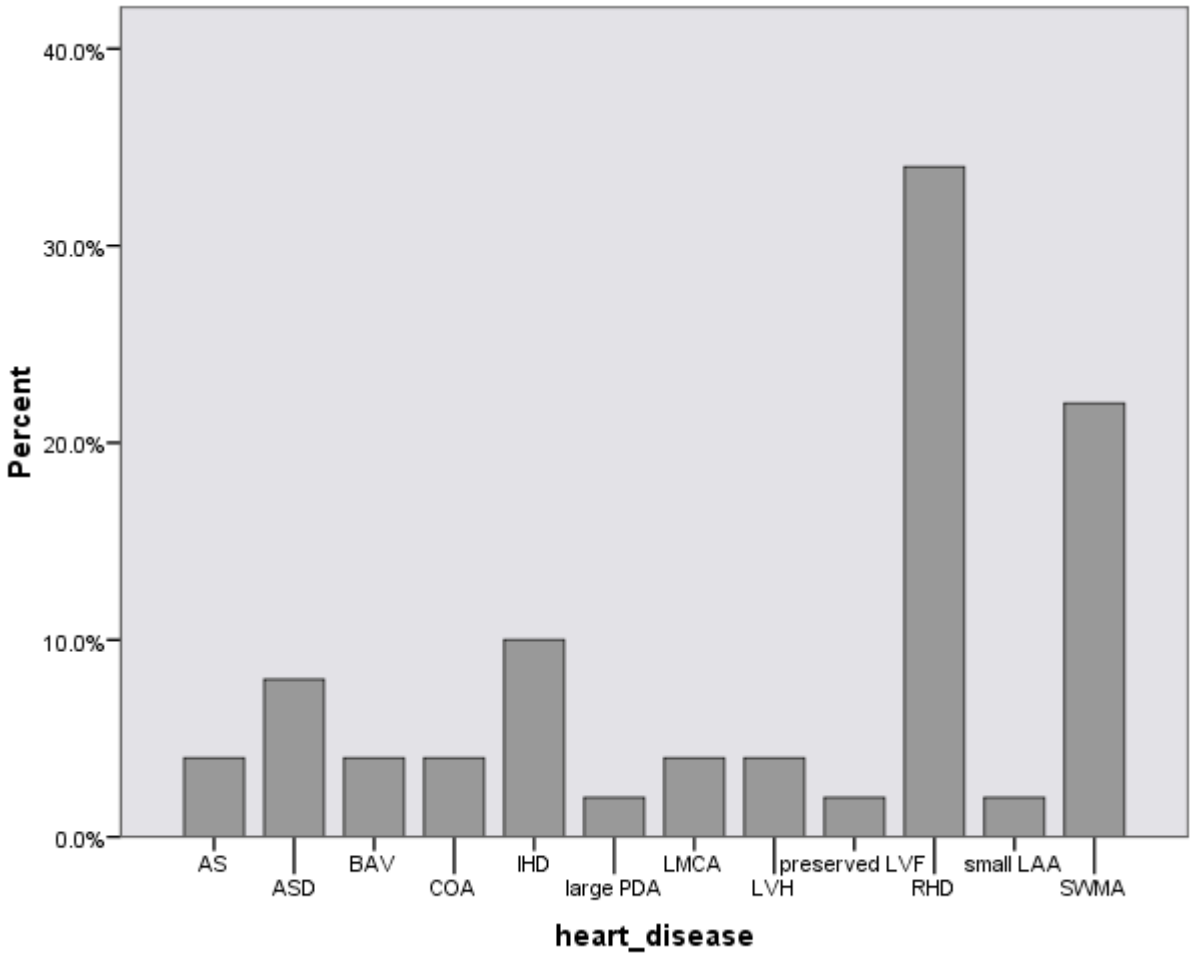


Figure 3: It shows the results of frequency table in graphical form. AS (aortic stenosis)=4.0%, ASD (atrial septal defect)=8.0%, BAV (bicuspid aortic valve)=4.0%, COA (coarctation of aorta)=4.0%, IHD (ischemic heart disease)=10.0%, large PDA (patent ducutus arteriosus)=2.0%, LMCA (stenosis of left main coronary artery)=4.0%, LVH (left ventricular hypertrophy)=4.0%, preserved LVF (left ventricular systolic function)=2.0%, RHD (rheumatic heart disease)=34.0%, small LAA (left atrial appendage)=2.0% and SWMA (segmental wall motion abnormalities)=22.0%.

Aortic stenosis is one of the most prevalent valve diseases and is a clinical progression of aortic sclerosis. It is the narrowing of aortic valve opening thus

restricting the blood flow and developing pressure in left atrium. The development of valvular stenosis is preceeded by valvular clacification however the limited genetic information is available regarding calcification of valves. Genome wide association studies performed with aortic valve calcification determined that genetic variations in LPA locus (rs10455872) are associated with aortic valve calcification hence leading to aortic stenosis (45). Atrial septal defect is the birth defect in which there is a hole in septum that divides atria of the heart. Some genes were comprehended to control the formation of atrial septum and familial ASD has been attributed to cardiac transcription factor mutations in past. However genome-wide linkage analysis characterized a novel ACTC1 mutation that is associated with late-onset dilated cardiomyopathy and ASD. Bioinformatics analysis identified deregulated genes in ASD. The cardiac sarcomeric proteins (MYL7, MYL3, MYH2, TNNT3 and TNNT1), extracellular signal molecules (BMP10 and VEGFA) and transcription factors (NKX2-5 and GATA4) were down regulated in ASD (46, 47).

Bicuspid aortic valve is ahighly heritable trait but the genetic elucidation remains largely elusive so more comprehensive studies are needed to elucidate the genetic connections. The only affirmed candidate gene connected with both sporadic and familial BAV is NOTCH1 (48). Coarctation of aorta is constriction in portion of an aorta thus forcing the blood to pump harder. The comparative genomic hybridization suggested that deregulation of FOXC1 play a crucial role in pathogenesis of COA (49). Ischemic heart disease (IHD) is the problem caused by narrowing of heart arteries. It is considered as highly polygenic disease. Meta-analysis of studies of genome wide association has showed nearest linked genes with IHD such as: PCSK9, SORT1, MRAS, KCNK5, NOS3 and CXCL12 (50).

Patent ductus arteriosus is a heart defect that can develop after birth affecting the flow of blood to the lungs of the baby. PDA is a compound disease with both environmental and genetic factors. Some of the specified genes that are associated with PDA and other genetic syndromes include SMADIP1, TGFBR2, TFAP2B and TBX5 (51). Left ventricular hypertrophy is a strong predictor for morbidity and mortality of cardiovascular disorders and several studies have indicated the control of genetic factors in LV mass. Genome wide association studies have successfully identified linkage of chromosome 7, 12 and 22 with increase in LV mass. The

angiotensin II type 1 gene A1166C and angiotensinogen gene M235T have been implicated in LV hyperthrophy induced by exercise (52). Transcriptome expression profile data and genome wide association studies on left atrial appendages identified two new genes MYPN and ERBB2 to be contributing towards the development of atrial appendage (53).

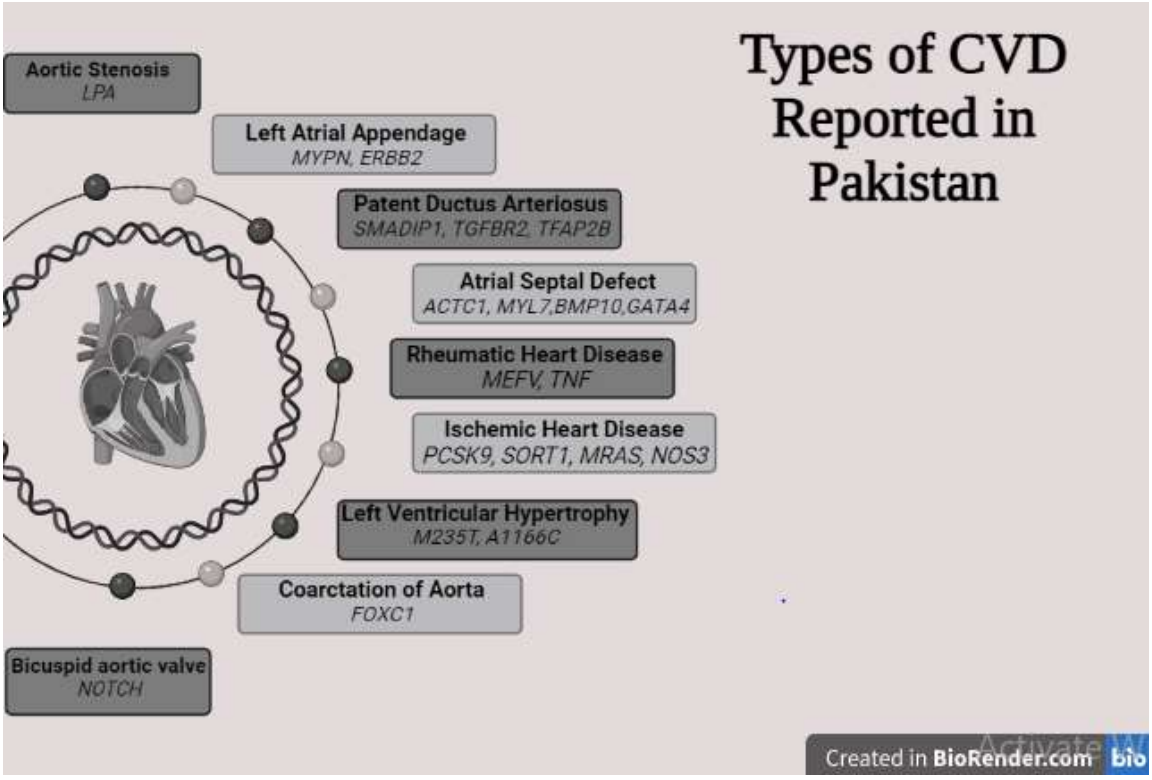


Figure 4: Types of Cardiovascular disorders reported in Pakistan and some of the reported associated specific genes

Discussion:

This review has systematically assessed the literature for genetic elucidation of cardiovascular disorders specifically in Pakistan. We have identified 49 papers from last 10 years that investigated genetic contributions towards the development of cardiovascular disorders and the associated risk factors in Pakistan. Cardiovascular disorder is a major causality of morbidity and mortality around the globe. However, the Asian region has a greater burden of CVD as compared to western population. CVD includes different types of disorders such as cardiomyopathy, coronary artery

disease, heart failure or peripheral arterial disease. The studies highlight some key areas, including the results of meta-analysis, studies of genome-wide association and whole exome sequencing showing the influence of genes as a risk factor for CVD. Systematic evaluation of studies was done to highlight the aspects of epigenetics in the development of CVD such as the role of BRCA1/2 in progression of the CVD disorder (16).

We have summarized the role of gene mutations leading to the development of cardiovascular disorders. CVD being a multifactorial disorder includes many gene mutations however mutation of 50 genes have been related with the development of cardiomyopathy (19). A study was included which identified the association between mutations in somatic cells and CVD in 160 genes however only 4 genes (TET2, DNMT3A, ASXL1 and JAK2) accounted for more than 70% mutations (21). The role of mitochondrial mutations on sex-specific cardiac functions was assessed showing that mitochondrial mutations are more deleterious in males (22). Other studies included showed mutations in different genes leading to the disruption of signaling pathways or protein functions hence causing cardiovascular disorders.

The CVD is a serious problem for Pakistan affecting both the genders however,we found little documented data on it and the role of consanguinity in the progression of cardiovascular disorders. The data we systematically collected showed 17.5% prevalence of CVD in Punjab among which 16.6% were male and 18.3% were females (8). This review article assessed the main risk factors for CVD in Pakistan which included family history, consanguinity, pollution and obesity. The genetic score of 2.96 for CVD risk in Pakistan was reported and a significant association with blood lipid levels was shown (37). Our study presented the novel variants reported in Pakistan in last 10 years. Novel mutation in genes MEFV, TNF, TNN13K, MYBPC3, mt-DNA encoded gene 16S-rRNA, CACNA1D and Prothrombin gene were reported.

The study included the analyses of total 50 cardiac reports from different healt institutes in Lahore, Pakistan. The results showed that Rheumatic heart disease (RHD) is the most prevalent cardiovascular disorder in Pakistani population with the

frequency of 34.0% whereas preserved LVF and PDA are the least reported cases with the frequency of 2.0%.

Conclusion:

Overall, our study contributes to the genetic spectrum of cardiovascular disorders in Pakistan. This will help the genetic counselors to find the novel gene variants for CVD present in Pakistani families and to compute the role of risk factors in advancement of this disorder. In future with the current advances in genetic screening and diagnosis it may be possible to diminish some of the adverse outcomes associated with consanguinity leading to rise of CVD.

References:

1. Genetic Disorders: What Are They, Types, Symptoms & Causes [cited 2021. Available from: https://my.clevelandclinic.org/health/diseases/21751-genetic-disorders.
2. Bick D, Bick SL, Dimmock DP, Fowler TA, Caulfield MJ, Scott RH. An online compendium of treatable genetic disorders. Am J Med Genet C Semin Med Genet. 2021;187(1):48-54.
3. Paone C, Diofano F, Park DD, Rottbauer W, Just S. Genetics of Cardiovascular Disease: Fishing for Causality. Front Cardiovasc Med. 2018;5:60.
4. Cardiovascular diseases (CVDs) [Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
5. Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. Nat Rev Genet. 2017;18(6):331-44.
6. Heart disease - Symptoms and causes [Available from: https://www.mayoclinic.org/diseases-conditions/heart-disease/symptoms-causes/syc-20353118.
7. Liaquat A, Javed Q. Current Trends of Cardiovascular Risk Determinants in Pakistan. Cureus. 2018;10(10):e3409.
8. Zubair F, Nawaz SK, Nawaz A, Nangyal H, Amjad N, Khan MS. Prevalence of cardiovascular diseases in Punjab, Pakistan: a cross-sectional study. Journal of Public Health. 2018;26(5):523-9.
9. Shukla H, Mason JL, Sabyah A. Identifying genetic markers associated with susceptibility to cardiovascular diseases. Future science OA. 2018;5(1):FSO350.
10. Haskell GT, Jensen BC, Samsa LA, Marchuk D, Huang W, Skrzynia C, et al. Whole exome sequencing identifies truncating variants in nuclear envelope genes in patients with cardiovascular disease. Circulation: Cardiovascular Genetics. 2017;10(3):e001443.

11. Singh TP, Field MA, Bown MJ, Jones GT, Golledge J. Systematic review of genome-wide association studies of abdominal aortic aneurysm. Atherosclerosis. 2021;327:39-48.
12. Gill D, Cameron AC, Burgess S, Li X, Doherty DJ, Karhunen V, et al. Urate, blood pressure, and cardiovascular disease: evidence from mendelian randomization and meta-analysis of clinical trials. Hypertension. 2021;77(2):383-92.
13. Efstathiadou A, Gill D, McGrane F, Quinn T, Dawson J. Genetically determined uric acid and the risk of cardiovascular and neurovascular diseases: a Mendelian randomization study of outcomes investigated in randomized trials. Journal of the American Heart Association. 2019;8(17):e012738.
14. Kei A, Koutsouka F, Makri A, Elisaf M. Uric acid and cardiovascular risk: What genes can say. International Journal of Clinical Practice. 2018;72(1):e13048.
15. Sumi MP, Mahajan B, Sattar RSA, Nimisha, Apurva, Kumar A, et al. Elucidation of Epigenetic Landscape in Coronary Artery Disease: A Review on Basic Concept to Personalized Medicine. Epigenetics Insights. 2021;14:2516865720988567.
16. Zhou S, Jin J, Wang J, Zhang Z, Huang S, Zheng Y, et al. Effects of breast cancer genes 1 and 2 on cardiovascular diseases. Current Problems in Cardiology. 2021;46(3):100421.
17. Kim MS, Kim WJ, Khera AV, Kim JY, Yon DK, Lee SW, et al. Association between adiposity and cardiovascular outcomes: an umbrella review and meta-analysis of observational and Mendelian randomization studies. European heart journal. 2021;42(34):3388-403.
18. Kodo K, Uchida K, Yamagishi H. Genetic and cellular interaction during cardiovascular development implicated in congenital heart diseases. Frontiers in Cardiovascular Medicine. 2021;8:170.
19. Tessadori F, Roessler HI, Savelberg SM, Chocron S, Kamel SM, Duran KJ, et al. Effective CRISPR/Cas9-based nucleotide editing in zebrafish to model human genetic cardiovascular disorders. Disease models & mechanisms. 2018;11(10):dmm035469.
20. Brigant B, Metzinger-Le Meuth V, Rochette J, Metzinger L. TRIMming down to TRIM37: relevance to inflammation, cardiovascular disorders, and cancer in MULIBREY nanism. International Journal of Molecular Sciences. 2018;20(1):67.
21. Fuster JJ, Walsh K. Somatic mutations and clonal hematopoiesis: unexpected potential new drivers of age-related cardiovascular disease. Circulation research. 2018;122(3):523-32.
22. Vona R, Ascione, Barbara, Malorni, Walter, Straface, Elisabetta. Mitochondria and Sex-Specific Cardiac Function. In: Kerkhof PLM, Miller, Virginia M., editor. Sex-Specific Analysis of Cardiovascular Function: Springer International Publishing; 2018. p. 241-56.
23. Salvarani N, Crasto S, Miragoli M, Bertero A, Paulis M, Kunderfranco P, et al. The K219T-Lamin mutation induces conduction defects through epigenetic inhibition of SCN5A in human cardiac laminopathy. Nature Communications. 2019;10(1).
24. Wilde AAM, Amin, Ahmad S. Clinical Spectrum of SCN5A Mutations.4(5):569-79.

25. Spinelli L, Imbriaco M, Nappi C, Nicolai E, Giugliano G, Ponsiglione A, et al. Early Cardiac Involvement Affects Left Ventricular Longitudinal Function in Females Carrying α-Galactosidase A Mutation. Circulation: Cardiovascular Imaging. 2018;11(4).
26. Meester JAN, Verstraeten A, Alaerts M, Schepers D, Van Laer L, Loeys BL. Overlapping but distinct roles for NOTCH receptors in human cardiovascular disease. Clinical Genetics. 2019;95(1):85-94.
27. Baig A. Obesity in Pakistan; Current and Future Perceptions. November 14, 2018.
28. Shahid SU, Shabana, Rehman A, Hasnain S. Role of a common variant of Fat Mass and Obesity associated (FTO) gene in obesity and coronary artery disease in subjects from Punjab, Pakistan: a case control study. Lipids in Health and Disease. 2016;15(1).
29. Ullah M, Husseni, Aisha, Mahmood, Syed Uzair. Consanguineous marriages and their detrimental outcomes in Pakistan: an urgent need for appropriate measures. December 23, 2017;5:1.
30. Malik MS, Afzal, Muhammad, Farid, Alveena, Khan, Fati Ullah, Mirza, Bushra, Waheed, Mohammad Tahir. Disease Status of Afghan Refugees and Migrants in Pakistan. 2019;7.
31. Shakir SK, Azizullah A, Murad W, Daud MK, Nabeela F, Rahman H, et al. Toxic metal pollution in Pakistan and its possible risks to public health. Reviews of Environmental Contamination and Toxicology Volume 242. 2016:1-60.
32. Rasool A, Farooqi A, Xiao T, Ali W, Noor S, Abiola O, et al. A review of global outlook on fluoride contamination in groundwater with prominence on the Pakistan current situation. Environmental Geochemistry and Health. 2018;40(4):1265-81.
33. Integration of AirQ⁺ and particulate matter mass concentration to calculate health and ecological constraints in Islamabad, Pakistan [Available from: https://ieeexplore.ieee.org/abstract/document/8667203/.
34. Shahid SU, Shabana, Cooper JA, Beaney KE, Li K, Rehman A, et al. Genetic risk analysis of coronary artery disease in Pakistani subjects using a genetic risk score of 21 variants. Atherosclerosis. 2017;258:1-7.
35. Shabana N, Ashiq S, Ijaz A, Khalid F, Saadat IU, Khan K, et al. 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. 2018;17(1).
36. Harshfield EL, Koulman A, Ziemek D, Marney L, Fauman EB, Paul DS, et al. An Unbiased Lipid Phenotyping Approach To Study the Genetic Determinants of Lipids and Their Association with Coronary Heart Disease Risk Factors. Journal of Proteome Research. 2019;18(6):2397-410.
37. Shahid SU, Shabana, Humphries S. The SNP rs10911021 is associated with oxidative stress in coronary heart disease patients from Pakistan. Lipids in Health and Disease. 2018;17(1).
38. Ramzan S, Tennstedt S, Tariq M, Khan S, Noor Ul Ayan H, Ali A, et al. A Novel Missense Mutation in TNNI3K Causes Recessively Inherited Cardiac Conduction Disease in a Consanguineous Pakistani Family. Genes. 2021;12(8):1282.

39. Shakeel M, Irfan M, Khan IA. Estimating the mutational load for cardiovascular diseases in Pakistani population. PLOS ONE. 2018;13(2):e0192446.
40. Shakeel M, Irfan, Muhammad, Khan, Ishtiaq Ahmad. Rare genetic mutations in Pakistani patients with dilated cardiomyopathy.673:134-9.
41. Rehman S, Ahmad N, Akhtar N, Usman S, Munir S. Mutation Screening of MEFV and TNF Gene in Pakistani Patients with Rheumatic Heart Disease: A Case Control-Study. J Clin Cell Immunol. 2015;6(382):2.
42. Khan M, Khan TA, Din AU, Khan MF, Ullah I, Ullah K, et al. Variants of MT-RNR2, MT-TI and MT-TL1 Genes in Hypertrophic Cardiomyopathy Families of Pakistan. Pakistan Journal of Zoology. 2020;52(6).
43. Liaqat K, Schrauwen I, Raza SI, Lee K, Hussain S, Chakchouk I, et al. Identification of CACNA1D variants associated with sinoatrial node dysfunction and deafness in additional Pakistani families reveals a clinical significance. Journal of human genetics. 2019;64(2):153-60.
44. Khan M, Altaf C, Saeed Malik H, Abdul Naeem M, Latif A. Heritable Thrombophilia in Venous Thromboembolism in Northern Pakistan: A Cross-Sectional Study. Advances in Hematology. 2021;2021:1-5.
45. Thanassoulis G, Campbell CY, Owens DS, Smith JG, Smith AV, Peloso GM, et al. Genetic associations with valvular calcification and aortic stenosis. N Engl J Med. 2013;368:503-12.
46. Wang W, Niu Z, Wang Y, Li Y, Zou H, Yang L, et al. Comparative transcriptome analysis of atrial septal defect identifies dysregulated genes during heart septum morphogenesis. Gene. 2016;575(2):303-12.
47. Frank D, Rangrez AY, Friedrich C, Dittmann S, Stallmeyer B, Yadav P, et al. Cardiac α-actin (ACTC1) gene mutation causes atrial-septal defects associated with late-onset dilated cardiomyopathy. Circulation: Genomic and Precision Medicine. 2019;12(8):e002491.
48. Andreassi MG, Della Corte A. Genetics of bicuspid aortic valve aortopathy. Current opinion in cardiology. 2016;31(6):585-92.
49. Sanchez-Castro M, Eldjouzi H, Charpentier E, Busson P-F, Hauet Q, Lindenbaum P, et al. Search for rare copy-number variants in congenital heart defects identifies novel candidate genes and a potential role for FOXC1 in patients with coarctation of the aorta. Circulation: Cardiovascular Genetics. 2016;9(1):86-94.
50. Elosua R, Sayols-Baixeras S. The genetics of ischemic heart disease: from current knowledge to clinical implications. Revista Española de Cardiología (English Edition). 2017;70(9):754-62.
51. Hajj H, Dagle JM, editors. Genetics of patent ductus arteriosus susceptibility and treatment. Seminars in perinatology; 2012: Elsevier.
52. Bella JN, Göring HH. Genetic epidemiology of left ventricular hypertrophy. American journal of cardiovascular disease. 2012;2(4):267.
53. Meng X, Nie Y, Wang K, Fan C, Zhao J, Yuan Y. Identification of Atrial Fibrillation-Associated Genes ERBB2 and MYPN Using Genome-Wide Association and Transcriptome Expression Profile Data on Left–Right Atrial Appendages. Frontiers in Genetics. 2021;12:696591.

5/13/2022 Turnitin

Biotechnology

About this page

This is your assignment inbox. To view a paper, select the paper's title. To view a Similarity Report, select the paper's Similarity Report icon in the similarity column. A ghosted icon indicates that the Similarity Report has not yet been generated.

Second Chance

Inbox | Now Viewing: new papers ▼

Submit File Online Grading Report | Edit assignment settings | Email non-submitters

Delete | Download | move to...

	Author	Title	Similarity	web	publication	student papers	Grade	response	File	Paper ID	Date
	Raiha Revised	Second chance	5% 5%	4%	2%	2%			download paper	1834504014	12-May-2022
	Shanzeh Shanzeh	Second chance	5% 5%	4%	2%	1%			download paper	1834504402	12-May-2022
	Azka Abid	BS Second chance	9% 9%	6%	6%	2%			download paper	1834503749	12-May-2022
	Eman Azhar	Second chance	15% 15%	12%	9%	2%			download paper	1834545694	12-May-2022
	Nabeeha Nabeeha	Second chance	15% 15%	8%	11%	0%			download paper	1834545061	12-May-2022
	Final Thesis_ Abeera...	Second chance	16% 16%	12%	12%	2%			download paper	1834503409	12-May-2022
	Rija Zainab	Second chance	16% 16%	12%	14%	3%			download paper	1834545381	12-May-2022