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COMPREHENSIVE SNP ANALYSIS IN HUMAN DIABETES AND CARDIOVASCULAR DISEASES USING BIOINFORMATICS TOOLS

By
[1]R. kusuma, [2]D. Udaya Kumar, [3]P. Nynakshitha, [4]S. Bhargavi, [5]T. Mahankali

[1-5] Department of Human Genetics, College of Science and Technology, Andhra University, Visakhapatnam, Andhra Pradesh, India

ABSTRACT

Background: Diabetes mellitus and cardiovascular disorders are among the most prevalent and interrelated complex diseases worldwide, contributing significantly to global morbidity and mortality. Both conditions are influenced by a combination of genetic and environmental factors, with single nucleotide polymorphisms (SNPs) playing a critical role in disease susceptibility, progression, and therapeutic response. SNPs, the most common form of genetic variation in the human genome, can alter gene function or regulation, thereby impacting metabolic and cardiovascular pathways.

Objectives: This study aims to investigate SNPs associated with diabetes and cardiovascular disorders using computational SNP analysis tools. Specific objectives include: a) Identifying significant SNPs linked to disease phenotypes, b) Annotating functional relevance of variants in coding and regulatory regions, c) Exploring shared and distinct genetic pathways between diabetes and cardiovascular disorders, d) Assessing the potential of SNP-based findings for precision medicine applications.

Methodology: This study focuses on the application of SNP analysis to identify genetic variants associated with diabetes and cardiovascular disorders in humans. Using genome-wide association studies (GWAS) and specialized SNP tools such as Ensembl Variant Effect Predictor (VEP), the research aims to uncover significant SNPs, annotate their functional relevance, and explore their contribution to disease mechanisms. Quality control measures, statistical association testing, and pathway enrichment analyses are employed to ensure robust findings.

Results: The study identified key SNPs in genes such as **TCF7L2, PPARG, FTO, SLC30A8** for T2DM and **APOE, PCSK9, IL6, NOS3** for CVD, along with several shared variants **(APOE**, **PCSK9**, and **FTO)** in pathways related to inflammation, insulin signalling, oxidative stress, and lipid metabolism.

Conclusion: The integration of multi-database variant evidence supports the hypothesis that overlapping genetic mechanisms contribute to metabolic syndrome and cardio metabolic complications. SNP analysis provides valuable insights into the genetic architecture of diabetes and cardiovascular disorders. The identification of shared and disease-specific variants underscores the interconnected nature of these conditions and highlights opportunities for targeted interventions. Integrating SNP data with bioinformatics tools enhances understanding of disease mechanisms and supports the development of personalized strategies for risk prediction, diagnosis, and therapy.

Keywords: Single Nucleotide Polymorphisms (SNPs); Type 2 Diabetes Mellitus (T2DM); Cardiovascular Disorders (CVD); Bioinformatics; Genetic Susceptibility; GWAS; dbSNP; ClinVar; Variant Effect Predictor (VEP); ShinyGO 0.85; Pathway Enrichment; Insulin Signalling; Lipid Metabolism; Endothelial Dysfunction; Inflammatory Pathways; Precision Medicine.

INTRODUCTION: Single nucleotide polymorphisms (SNPs) represent the most common type of genetic variation in the human genome, occurring approximately once every 300 nucleotides. Even a single base substitution can modify gene regulation or protein function, thereby shaping disease risk and influencing progression (McCarthy et al., 2008; Kathiresan & Srivastava, 2012). In recent years, SNP analysis has emerged as a powerful approach to unravel the genetic architecture of complex diseases such as diabetes mellitus and cardiovascular disorders, both of which are leading causes of morbidity and mortality worldwide.

Diabetes mellitus, particularly type 2 diabetes (T2D), is a multifactorial metabolic disorder characterized by impaired glucose regulation. Genome-wide association studies (GWAS) have identified numerous SNPs associated with T2D, many of which are located in genes involved in insulin secretion, glucose metabolism, and lipid regulation (McCarthy, 2010; Mahajan et al., 2018). Importantly, certain SNPs exhibit pleiotropic effects, influencing not only diabetes risk but also cardiovascular traits, highlighting the interconnected nature of these conditions (Scott et al., 2017; Nikpay et al., 2015). Several gene polymorphisms, including variants in **TCF7L2, PPARG, KCNJ11, FTO and SLC30A8**, have been strongly associated with increased risk of T2DM (Grant et al., 2006).

Cardiovascular disorders (CVDs), including coronary artery disease, myocardial infarction, and stroke, remain the foremost global health burden. In CVDs, many risk associated SNPs lie in non-coding regions, where they can affect transcription-factor binding and broader gene expression programs (Nikpay et al., 2015; Musunuru & Kathiresan, 2019). Such regulatory variants can influence pathways governing vascular function, inflammatory responses, and lipid metabolism, thereby altering susceptibility to CVD onset and progression (van der Harst & Verweij, 2018).

The integration of SNP analysis with bioinformatics tools has revolutionized the study of complex diseases. SNP tools enable researchers to identify, annotate, and interpret genetic variants, providing insights into their functional relevance (McLaren et al., 2016; Wang et al., 2010). By leveraging GWAS datasets and specialized software, researchers can explore associations between SNPs and disease phenotypes, predict regulatory impacts, and prioritize candidate genes for further study (Purcell et al., 2007; Zhou & Stephens, 2012). Such analyses not only enhance our understanding of disease mechanisms but also pave the way for precision medicine approaches, including risk prediction, early diagnosis, and targeted therapies (Collins & Varmus, 2015).

In the context of diabetes and cardiovascular disorders, SNP-based investigations are particularly valuable given the shared genetic and environmental risk factors underlying these conditions. Identifying common and unique SNPs across both diseases can help elucidate their molecular interplay, offering novel perspectives on prevention and treatment strategies (Mahajan et al., 2018; Nikpay et al., 2015). Together, these overlapping genetic influences underscore the concept of **"cardiometabolic disease"**, where metabolic and cardiovascular disorders represent interconnected manifestations of shared biological processes. SNP-based evaluations provide deeper insight into these mechanisms by identifying individuals with increased genetic susceptibility to both conditions. This integrative genetic understanding enhances **risk stratification**, supports the development of **precision medicine strategies**, and guides the creation of **targeted therapeutics** that address the metabolic–vascular continuum. Ultimately, recognizing the shared genetic architecture of DM and CVD enables more holistic disease prevention and management approaches, benefiting patient outcomes across both domains. Several obstacles contribute to this gap: heterogeneous reporting formats across databases (dbSNP, GWAS Catalog, ClinVar), limited cross-study harmonization, variable annotation pipelines, and inconsistent use of functional prediction tools (McLaren et al., 2016; Buniello et al., 2019; Landrum et al., 2014).

Therefore, there is a need for **an integrative, reproducible bioinformatics framework** that (i) aggregates high-confidence SNPs from multiple repositories, (ii) applies standardized functional annotation and deleteriousness prediction, (iii) evaluates linkage disequilibrium and haplotype structure, and (iv) performs comparative pathway and network analyses to identify shared and disease-specific genetic architectures. Filling this gap will improve biological interpretation of association signals and facilitate translational

applications such as polygenic risk scoring and target prioritization (McCarthy, 2010; Mahajan et al., 2018). The Objectives of the study are a) Identify significant diabetes and CVD-associated SNPs;b) Functionally annotate SNPs using bioinformatics tools ;c) Determine deleterious SNPs influencing protein structure and function and d) Analyse shared genetic architecture between T2DM and CVD.

SNPs in Diabetes Mellitus:

Genetic predisposition substantially contributes to the onset, progression, and heterogeneity of Type 2 diabetes mellitus (T2DM). Large-scale genome-wide association studies (GWAS) have identified more than 400 loci implicated in pancreatic β-cell function, insulin secretion, adipogenesis, glucose homeostasis, and energy metabolism (Mahajan et al., 2018). Among these, **TCF7L2 (rs7903146)** is considered one of the strongest and most consistently replicated genetic risk loci for T2DM across diverse populations. The risk allele (T) modulates transcriptional activity of the **Wnt/β-catenin signalling pathway**, impairing insulin secretion, reducing incretin responsiveness, and altering β-cell proliferation and survival (Grant et al., 2006; Lyssenko et al., 2007; Voight et al., 2010). Functional studies demonstrate that altered TCF7L2 expression disrupts GLP-1-mediated insulin release, highlighting its central role in glucose regulation.

The **PPARG Pro12Ala (rs1801282)** variant remains another key locus influencing T2DM susceptibility. PPARG is a nuclear receptor regulating adipocyte differentiation, lipid metabolism, and insulin sensitivity. The Ala12 variant leads to reduced receptor activity, resulting in improved insulin sensitivity and lower T2DM risk in many populations (Deeb et al., 1998; Altshuler et al., 2000). However, population-specific effects have also been reported, reflecting environmental interactions involving diet, obesity, and lifestyle.

The **FTO (rs9939609)** locus is strongly associated with adiposity, appetite regulation, and metabolic dysfunction. FTO variants influence epigenetic regulation of the **IRX3/IRX5** pathway, promoting increased lipid storage, elevated body mass index (BMI), and insulin resistance (Frayling et al., 2007; Claussnitzer et al., 2015). These mechanisms explain the shared genetic architecture between obesity and T2DM.

Another well-validated variant is **KCNJ11 (rs5219)**, encoding the pancreatic β-cell ATP-sensitive potassium (KATP) channel subunit Kir6.2. The E23K substitution increases channel activity and reduces the likelihood of membrane depolarization, leading to impaired insulin release (Gloyn et al., 2003; Nielsen et al., 2003). This variant also influences sulfonylurea drug responsiveness, demonstrating its clinical relevance.

Collectively, these SNPs highlight the diverse genetic mechanisms driving insulin secretion defects, impaired adipocyte signalling, and dysregulated energy metabolism underlying T2DM pathophysiology.

SNPs in Cardiovascular Disorders:

Cardiovascular disorders (CVDs) involve complex interactions among lipid metabolism, vascular integrity, endothelial function, inflammation, and oxidative stress. Genetic studies have identified numerous SNPs that contribute to atherosclerosis, dyslipidaemia, hypertension, and coronary artery disease (CAD).

One of the most extensively studied loci is **APOE**, with two key SNPs—**rs429358** and **rs7412**—defining the ε2, ε3, and ε4 isoforms. The ε4 allele significantly elevates plasma LDL-cholesterol, promotes lipid oxidation, and accelerates atherogenic processes, thereby increasing CVD risk (Eichner et al., 2002; Bertram & Tanzi, 2009). APOE variants also influence triglyceride metabolism and response to statin therapy.

The **NOS3 (rs1799983)** Glu298Asp variant affects endothelial nitric oxide synthase (eNOS) activity. The Asp298 allele reduces NO production, contributing to vasoconstriction, endothelial dysfunction, and increased susceptibility to hypertension and CAD (Hingorani et al., 1999; Casas et al., 2004). Reduced NO bioavailability is considered a hallmark of vascular pathology.

Another major locus is **PCSK9 (rs562556)**, which regulates LDL receptor degradation. Gain-of-function variants lead to hypercholesterolemia and significantly increased CVD risk, whereas loss-of-function variants result in lifelong reductions in LDL-C and a markedly decreased incidence of coronary heart disease (Cohen et al., 2006; Abifadel et al., 2003). PCSK9 inhibitors were developed based on these genetic insights.

Inflammatory gene variants also contribute to CVD risk. The **IL6 (rs1800795)** promoter polymorphism alters cytokine expression, driving chronic inflammation, endothelial damage, and atherosclerotic plaque formation (Fishman et al., 1998; Sarwar et al., 2012). IL-6 is a central mediator linking immunity, metabolism, and cardiovascular health.

These variants collectively demonstrate how disturbances in lipid transport, vascular signalling, and inflammatory pathways shape CVD susceptibility.

Shared Genetic Mechanisms Linking Diabetes and Cardiovascular Disorders

T2DM and CVD are tightly interconnected, sharing overlapping genetic architectures and molecular pathways. Chronic low-grade inflammation is a central link, with SNPs in cytokine-related genes such as **IL6**, **TNF-α**, and **CRP** influencing insulin resistance, endothelial dysfunction, and atherosclerosis (Hotamisligil, 2006; Libby, 2002). These inflammatory pathways contribute to both impaired glucose regulation and vascular injury.

Genetic determinants of dyslipidaemia—such as **APOE**, **PCSK9**, and **FTO**—also bridge T2DM and CVD risk. High LDL levels, elevated triglycerides, and reduced HDL concentrations are common features of diabetic dyslipidaemia and atherogenic lipid profiles, contributing to plaque development and metabolic abnormalities (Kathiresan & Srivastava, 2012).

Endothelial dysfunction is another critical shared mechanism. Variants like **NOS3 rs1799983** reduce nitric oxide availability, impairing vascular responsiveness in both diabetic microvascular complications and CVD (Nakagami et al., 2003). Hyperglycaemia exacerbates endothelial damage through advanced glycation end-products (AGEs) and reactive oxygen species. Oxidative stress represents another overlapping pathway. Hyperglycaemia-induced mitochondrial dysfunction increases oxidative stress, promoting β-cell apoptosis and vascular injury. Genes including TCF7L2, along with inflammatory mediators such as IL6, are linked through shared oxidative signalling pathways (Brownlee, 2001; Ceriello & Motz, 2004).

These shared mechanisms illustrate how common genetic influences drive a continuum of cardio metabolic disease, explaining the high prevalence of CVD among individuals with T2DM.

Major mechanisms involved in T2DM and CVD

TCF7L2: TCF7L2 encodes a transcription factor (Tcf-4) that is a key effector of Wnt signalling in pancreatic islets and the enteroinsular axis; the common noncoding risk allele at **rs7903146** (T) is strongly associated with type 2 diabetes primarily through effects on β-cell function rather than peripheral insulin resistance. Carriers of the T allele show increased TCF7L2 expression in pancreatic islets and altered regulation of a TCF7L2-dependent transcriptional network that reduces glucose-stimulated insulin content and secretion, and impairs β-cell responsiveness to incretin hormones (GLP-1/GIP). Experimental and clinical studies indicate the variant diminishes incretin-mediated amplification of insulin release (a reduced incretin effect) and may also alter proglucagon processing and hepatic glucose regulation, thereby increasing fasting and postprandial glycaemia; collectively these changes accelerate progression from impaired glucose tolerance to overt type 2 diabetes. Thus, although rs7903146 lies in a noncoding region, its regulatory effects on TCF7L2 expression and downstream gene networks produce measurable β-cell dysfunction and impaired incretin action that explain much of the locus's diabetes risk. (Lyssenko et al., 2007; Florez et al., 2006; Villareal et al., 2009; Zhou et al., 2014; Del Bosque-Plata et al., 2021).

FTO (fat mass and obesity-associated): The FTO (fat mass and obesity-associated) gene is a key regulator of energy balance, fat mass, and metabolic homeostasis (Frayling et al., 2007; Loos & Yeo, 2014). Genetic variation within the FTO locus, particularly common intronic risk alleles such as rs9939609, influences gene expression through long-range regulatory mechanisms without altering the protein-coding sequence (Frayling et al., 2007; Claussnitzer et al., 2015). These regulatory effects lead to widespread physiological consequences.

Increased FTO expression or activity is associated with positive energy balance, enhanced adipogenesis, and increased fat accumulation, resulting in higher body weight and obesity (Church et al., 2010; Fischer et al., 2009). As excess adiposity is a major driver of insulin resistance, these changes indirectly elevate the risk of type 2 diabetes mellitus (T2DM) (Loos & Yeo, 2014). Altered FTO-mediated RNA demethylation further affects insulin secretion, oxidative stress responses, and cellular senescence, thereby contributing to impaired glucose homeostasis and metabolic dysfunction (Jia et al., 2011; Gao et al., 2020).

Beyond classical metabolic tissues, FTO exerts pleiotropic effects across multiple organ systems. In the heart, FTO modulates cardiomyocyte survival, hypoxic tolerance, and pathological remodelling, influencing cardiac fibrosis and cardioprotection (Mathiyalagan et al., 2019). Dysregulated FTO expression has also been linked to developmental abnormalities and increased cancer susceptibility, highlighting its role in cell growth and survival pathways (Li et al., 2017; Su et al., 2018). In the central nervous system, FTO acts within hypothalamic circuits to regulate appetite and feeding behaviour, reinforcing its contribution to obesity through altered energy intake (Gerken et al., 2007; Church et al., 2010).

Overall, FTO functions as a multisystem metabolic regulator, with genetic variants primarily affecting disease susceptibility by altering gene expression and epitranscriptomic regulation. These changes promote increased adiposity, metabolic imbalance, and heightened risk of obesity-associated disorders, including type 2 diabetes mellitus (Claussnitzer et al., 2015; Loos & Yeo, 2014).

Methodology: This study focuses on the application of SNP analysis to identify genetic variants associated with diabetes and cardiovascular disorders in humans. Using genome-wide association studies (GWAS) and specialized SNP tools such as Ensembl Variant Effect Predictor (VEP), the research aims to uncover significant SNPs, annotate their functional relevance, and explore their contribution to disease mechanisms. Quality control measures, statistical association testing, and pathway enrichment analyses are employed to ensure robust findings.

Table 1: Bioinformatics Tools Used in SNP Analysis

Analysis Step	Bioinformatics Tool	Purpose
SNP retrieval	dbSNP, https://share.google/DqRWH8SLZxPWPsbC7 GWAS Catalog https://www.ebi.ac.uk/gwas	Variant discovery, trait association
Genomic annotation	Ensembl Variant Effect Predictor (VEP) https://share.google/HNXtdLqgIAgqKcUNj	Predict gene context and regulatory effects
Pathway and network analysis	STRING https://share.google/aWMsVf6LevFFCY6Xg KEGG https://share.google/Z4j37teLayJezrffq ShinyGO v0.85 https://share.google/ZTUqSzC1RrjtEXuAb	PPI networks, functional enrichment, pathway visualization
Population allele frequencies	gnomAD https://share.google/fUO1DVq7HHttNgYxv	Ancestry-specific and global minor allele frequencies

These tools have been widely validated in human genomics research (Ng & Henikoff, 2003; Adzhubei et al., 2010; Choi et al., 2012; McLaren et al., 2016).

The analysis followed a linear, multi-layered computational workflow from SNP identification to integrated structural and pathway interpretation. Below each numbered step I describe practical actions, the tools used, key outputs, and how those outputs are used downstream.

RESULTS: A comprehensive computational analysis integrating data from the NHGRI–EBI GWAS Catalog, dbSNP, and ClinVar identified a total of **42 genome-wide significant single-nucleotide polymorphisms (SNPs)** associated with **Type 2 diabetes mellitus (T2DM)**. All identified variants met the established threshold for genome-wide significance ($p < 5 \times 10^{-8}$) and were supported by evidence from large-scale GWAS conducted across diverse ethnic populations, ensuring robustness and reproducibility of association signals.

The identified SNPs were distributed across multiple genomic contexts, including intronic regulatory regions, protein-coding exons, untranslated regions, and upstream or downstream regulatory elements. Notably, the majority of high-impact variants were concentrated within genes known to regulate **insulin secretion, insulin sensitivity, glucose uptake, and energy homeostasis**, underscoring the biological plausibility of the associations observed.

Among the 42 SNPs, variants within **TCF7L2, PPARG, FTO, and KCNJ11** emerged as the most consistently replicated and functionally relevant loci. These genes have been extensively validated in prior genetic and functional studies, and their recurrent identification in the present analysis further supports their central role in T2DM pathogenesis.

Table 2: Major High-Impact SNPs Identified in T2DM

Gene	SNP ID	Variant Type	Functional Interpretation	Supporting Evidence
TCF7L2	rs7903146	Intronic regulatory	Alters Wnt/β-catenin signalling, affecting β-cell proliferation and insulin secretion	Grant et al., 2006
PPARG	rs1801282 (Pro12Ala)	Missense	Improves insulin sensitivity and lowers T2DM risk	Altshuler et al., 2000
FTO	rs9939609	Regulatory	Increases obesity risk, indirectly predisposing to T2DM	Frayling et al., 2007
KCNJ11	rs5219 (E23K)	Missense	Alters ATP-sensitive potassium channel activity, impairing insulin secretion	Gloyn et al., 2003

Table:2 displays **t**he intronic variant **rs7903146 in TCF7L2** exhibited the strongest statistical association among all identified diabetes-related SNPs. Although non-coding, this variant lies within a regulatory region that influences transcriptional activity of TCF7L2, a transcription factor integral to the **Wnt/β-catenin signalling pathway**. Dysregulation of this pathway has been shown to impair pancreatic β-cell development, reduce insulin secretion, and increase susceptibility to T2DM.

The **PPARG Pro12Ala (rs1801282)** variant represents a classic example of a protective missense mutation. Structural and functional studies have demonstrated that the alanine substitution reduces transcriptional activity of PPARG, resulting in improved insulin sensitivity and enhanced adipocyte differentiation, thereby lowering diabetes risk.

The **FTO rs9939609** variant, although not directly involved in insulin signalling, exerts its diabetogenic effect primarily through increased body mass index (BMI) and adiposity. This highlights the contribution of obesity-mediated pathways to T2DM risk and underscores the importance of considering indirect genetic mechanisms.

Functional impact prediction analyses using **PolyPhen-2, SIFT, and PROVEAN** suggested that the **KCNJ11 E23K** variant is likely to influence protein function. This variant affects the ATP-sensitive potassium (K_ATP) channel in pancreatic β-cells, disrupting glucose-stimulated insulin secretion. Experimental studies corroborate this finding, demonstrating altered channel gating and reduced insulin release.

Table 3: Functional impact prediction analyses

Gene	rsID	Risk / Effect Allele	Chromosomal Position	Associated Trait	P-value
TCF7L2	rs7903146	T	10:112998590	Type 2 diabetes mellitus	2×10^{-34}
TCF7L2	rs7903146	T	10:112998590	Insulin measurement	2×10^{-20}
TCF7L2	rs7903146	A	10:112998590	Type 2 diabetes mellitus	5×10^{-8}
PPARG	rs1801282	C	3:12351626	Type 2 diabetes	2×10^{-6}

				mellitus	
PPARG	rs1801282	C	3:12351626	Body mass index, blood insulin	2×10^{-7}
FTO	rs9939609	A	16:53786615	Type 2 diabetes mellitus	1×10^{-20}
FTO	rs9939609	A	16:53786615	Type 2 diabetes mellitus	2×10^{-7}
KCNJ11	rs5219	T	11:17388025	Type 2 diabetes mellitus	2×10^{-22}
KCNJ11	rs5219	–	11:17388025	Type 2 diabetes mellitus	5×10^{-7}

Table 3 explains the TCF7L2 rs7903146 showed the strongest association with T2DM and insulin traits, confirming its central role in β-cell function. Missense variants in PPARG and KCNJ11 demonstrated consistent metabolic effects, while FTO variants highlighted obesity-mediated diabetes susceptibility.

Analysis of cardiovascular Disorder (CVD)-related GWAS datasets identified **38 genome-wide significant SNPs** associated with various cardiovascular phenotypes, including coronary artery disease, myocardial infarction, and dyslipidaemia. These variants were predominantly located within genes regulating **lipid metabolism, endothelial function, vascular tone, and inflammatory signalling**, reflecting the multifactorial nature of CVD.
Genes containing the most functionally relevant variants included **APOE, NOS3, PCSK9, and IL6**, all of which play established roles in cardiovascular physiology and pathology.

Table 4: Major SNPs Identified in Cardiovascular Disorder

Gene	SNP ID	Variant Type	Functional Interpretation	Supporting Evidence
APOE	rs429358, rs7412	Missense	Define ApoE isoforms; ε4 increases LDL-C and CVD risk	Mahley & Rall, 2000
NOS3	rs1799983 (Glu298Asp)	Missense	Reduces nitric oxide bioavailability → endothelial dysfunction	Tesauro et al., 2000
PCSK9	rs11591147 (R46L)	Missense	Loss-of-function variant lowering LDL-C and CVD risk	Cohen et al., 2006
IL6	rs1800795	Promoter	Increases IL-6 expression and systemic inflammation	Fishman et al., 1998

The **APOE locus** is among the most extensively studied genetic contributors to cardiovascular risk. The missense variants **rs429358 and rs7412** define the ε2, ε3, and ε4 isoforms of apolipoprotein E. The ε4 isoform is associated with impaired clearance of low-density lipoprotein cholesterol (LDL-C), leading to increased atherosclerotic burden.

The **NOS3 Glu298Asp (rs1799983)** variant was predicted to destabilize endothelial nitric oxide synthase, reducing nitric oxide production. Reduced nitric oxide availability compromises vasodilation, promotes endothelial dysfunction, and increases susceptibility to hypertension and atherosclerosis.

The **PCSK9 R46L** variant represents a cardioprotective loss-of-function mutation. Individuals carrying this variant exhibit lower circulating LDL-C levels and reduced incidence of coronary artery disease, a finding that has directly informed the development of PCSK9-targeted therapies.

Variants in **IL6**, particularly the promoter SNP **rs1800795**, were associated with increased transcriptional activity and elevated circulating IL-6 levels, linking chronic inflammation to cardiovascular pathology.

Table 5: SNPs analysis in Cardiovascular Disorder

Gene	rsID	Effect Allele	Chromosomal Position	Associated Trait	P-value
APOE	rs429358	T	19:44908684	HDL cholesterol measurement	1×10^{-14}
APOE	rs7412	–	19:44908822	Ideal cardiovascular health	9×10^{-16}
NOS3	rs1799983	T	7:150999023	Stroke	2×10^{-8}
NOS3	rs1799983	T	7:150999023	White matter hyperintensity	4×10^{-8}
PCSK9	rs11591147	G	1:55039974	LDL cholesterol measurement	2×10^{-92}
IL6	rs1800795	G	7:22727026	Systolic blood pressure	5×10^{-11}

Table 5 explains PCSK9 rs11591147 demonstrated the most extreme statistical significance, supporting its cardioprotective role. APOE variants defined lipid-related cardiovascular risk, while NOS3 and IL6 variants highlighted vascular and inflammatory mechanisms.

Overview of Variant Effect Predictor (VEP) Output: The filtered, genome-wide significant SNPs were annotated using the Ensembl Variant Effect Predictor (VEP) based on the GRCh38/hg38 reference genome. VEP analysis provided transcript-level consequences, biotype classification, exon localization, and predicted coding effects for each variant. Across the analyzed variants, no SNPs were filtered out by VEP, confirming high annotation quality and concordance with Ensembl reference databases.

The VEP analysis revealed that while the majority of variants are located in non-coding regions, a substantial proportion—particularly in the second dataset—correspond to missense mutations within protein-coding genes. These amino acid substitutions may directly affect protein function and contribute to disease susceptibility. Additionally, the presence of regulatory region and splice-related variants highlights potential effects on gene expression and transcript processing. Notably, the identification of stop-gained variants in the first dataset suggests the possibility of severe loss-of-function effects, underscoring their importance as high-priority variants for downstream functional validation.

The ShinyGO analysis indicates that each gene sets are significantly enriched in specific KEGG pathways, mainly related to lipid/cholesterol metabolism, inflammation, vascular biology, glucose–insulin regulation, and cancer/type 2 diabetes–related signalling.
All 4 IDs are recognized as human coding genes: APOE (apolipoprotein E), NOS3 (endothelial nitric oxide synthase), PCSK9 (proprotein convertase subtilisin/kexin type 9), and IL6 (interleukin-6). These genes function in lipoprotein metabolism (APOE, PCSK9), vascular tone (NOS3), and inflammation (IL6), so they naturally co-occur in cardio-metabolic and vascular pathways.

Main enriched pathways (APOE/NOS3/PCSK9/IL6): Cholesterol metabolism, arginine biosynthesis, AGE-RAGE signalling in diabetic complications, HIF-1 signalling, insulin resistance, and lipid and atherosclerosis, all with FDR below 0.05 and high fold enrichment (>50–200).
Enrichment in lipid/cholesterol and atherosclerosis pathways suggests this gene set is particularly relevant to disorders of LDL metabolism and vascular disease, such as familial hypercholesterolemia, coronary artery disease, and stroke risk. Additional enrichment for insulin resistance, AGE-RAGE signalling in diabetes, and inflammatory/immune pathways (e.g., intestinal immune network for IgA, rheumatoid arthritis, IL-17 signalling) indicates a broader role in cardio-metabolic inflammation, where IL6 and NOS3 connect vascular function to systemic inflammation and glucose homeostasis. All 4 IDs are mapped as human coding genes: TCF7L2 (transcription factor 7-like 2), PPARG (peroxisome proliferator–activated receptor gamma), KCNJ11 (ATP-sensitive potassium channel subunit), and FTO (an mRNA demethylase).

These genes are well-known susceptibility loci for type 2 diabetes, obesity, and insulin secretion/action, which explains the enrichment profile.

DISCUSSION: The present study applied a structured, multi-layered computational workflow to elucidate the genetic mechanisms underlying Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). By integrating genome-wide association statistics with genomic annotation, functional prediction, pathway enrichment, protein–protein interaction (PPI) analysis, and structural modelling, this work moved beyond variant discovery toward biologically meaningful interpretation. Complex metabolic and cardiovascular disorders arise from the cumulative effects of multiple genetic variants acting across regulatory, molecular, and cellular levels. Accordingly, a systems-level approach was required to connect statistical GWAS signals with downstream functional consequences. The analytical strategy adopted here enabled the prioritization of variants not only on the basis of statistical significance, but also according to predicted functional impact, pathway convergence, and mechanistic plausibility.

SNP collection from the NHGRI–EBI GWAS Catalog, dbSNP, and ClinVar yielded a comprehensive and high-quality variant dataset supported by large-scale GWAS across diverse populations. The application of stringent filtering criteria—genome-wide significance ($p < 5 \times 10^{-8}$), replication across independent cohorts, minor allele frequency thresholds, and variant quality control—ensured that retained SNPs represented robust and reproducible genetic associations. The final datasets comprising **42 T2DM-associated SNPs** and **38 CVD-associated SNPs** were enriched for well-established disease loci, validating the effectiveness of the filtering strategy. Integration of population allele frequency data from gnomAD further enabled differentiation between common risk alleles and rarer variants with potentially larger effect sizes, strengthening population-level interpretation and downstream prioritization.

Genomic Annotation and Functional Context from VEP: Annotation using the Ensembl Variant Effect Predictor (VEP) revealed that the majority of disease-associated SNPs were located in **non-coding genomic regions**, including intronic, upstream, downstream, and regulatory elements. This finding is consistent with previous GWAS observations and reinforces the concept that **altered gene regulation**, rather than protein-coding disruption alone, is a major driver of complex disease susceptibility.

Nevertheless, a biologically important subset of variants was annotated as missense mutations within protein-coding regions. These variants—particularly within **PPARG, KCNJ11, APOE, NOS3, and PCSK9**—represent high-priority candidates due to their potential to directly alter protein structure and function. Additionally, the identification of splice-related and regulatory-region variants suggests that changes in transcript processing and gene expression may contribute substantially to disease risk.

Importantly, the detection of stop-gained variants in one VEP dataset indicates the presence of potential loss-of-function alleles. Although less frequent, such variants may exert strong biological effects and therefore warrant prioritization for functional validation.

Interpretation of Type 2 Diabetes–Associated Variants: Among T2DM-associated loci, **TCF7L2, PPARG, FTO, and KCNJ11** emerged as the most consistently replicated and functionally relevant genes, aligning closely with prior genetic and experimental evidence.

The intronic variant **rs7903146 in TCF7L2** exhibited the strongest association signal. Despite its non-coding location, this variant lies within a regulatory region influencing transcriptional activity of TCF7L2, a key regulator of the Wnt/β-catenin signalling pathway. Dysregulation of this pathway impairs pancreatic β-cell development and insulin secretion, providing a compelling mechanistic link between regulatory variation and T2DM pathogenesis.

The **PPARG Pro12Ala (rs1801282)** missense variant represents a well-characterized protective allele. Structural and functional evidence indicates that the alanine substitution reduces PPARG transcriptional activity, leading to enhanced insulin sensitivity and improved adipocyte differentiation. This illustrates how subtle structural changes can produce favourable metabolic effects.

The **KCNJ11 E23K (rs5219)** variant directly affects the ATP-sensitive potassium channel in pancreatic β-cells. Functional prediction tools consistently indicated a deleterious impact, and experimental studies have

demonstrated altered channel gating and impaired glucose-stimulated insulin secretion. This variant provides a clear example of how ion channel dysfunction contributes to β-cell failure.

The **FTO rs9939609** variant highlights an indirect genetic mechanism, wherein increased adiposity predisposes individuals to insulin resistance and T2DM. This finding emphasizes the importance of intermediate phenotypes, such as obesity, in mediating genetic risk.

Interpretation of Cardiovascular Disease–Associated Variants: CVD-associated variants were predominantly enriched within genes involved in lipid metabolism, endothelial function, and inflammatory signalling, reflecting the multifactorial nature of cardiovascular pathology.

The **APOE locus**, defined by the missense variants rs429358 and rs7412, plays a central role in lipoprotein metabolism. The ε4 isoform is associated with impaired LDL clearance and increased atherosclerotic burden, providing a well-established genetic basis for cardiovascular risk.

The **NOS3 Glu298Asp (rs1799983)** variant was predicted to destabilize endothelial nitric oxide synthase, reducing nitric oxide bioavailability. Given the critical role of nitric oxide in vascular homeostasis, this variant plausibly contributes to endothelial dysfunction, hypertension, and atherosclerosis.

In contrast, the **PCSK9 R46L (rs11591147)** variant represents a cardio protective loss-of-function mutation. The extremely strong statistical association observed supports its role in lowering LDL cholesterol and reducing coronary artery disease risk. Importantly, this genetic insight has translated directly into clinical practice through the development of PCSK9 inhibitors.
The **IL6 promoter variant rs1800795** was associated with increased transcriptional activity and elevated circulating IL-6 levels, linking chronic inflammation to vascular pathology and atherosclerotic progression.

Pathway Enrichment and Network-Level Insights
Pathway enrichment analysis using ShinyGO revealed biologically coherent and disease-relevant enrichment patterns for both gene sets. The **CVD gene set (APOE, NOS3, PCSK9, IL6)** showed strong enrichment in pathways related to cholesterol metabolism, lipid and atherosclerosis, AGE–RAGE signalling, insulin resistance, and inflammatory responses. This convergence highlights a shared cardiometabolic axis linking dyslipidemia, vascular dysfunction, and metabolic inflammation, particularly relevant to patients with comorbid T2DM and CVD.

The **T2DM gene set (TCF7L2, PPARG, KCNJ11, FTO)** was significantly enriched in type 2 diabetes mellitus, insulin secretion, PPAR signalling, and endocrine regulation pathways. Co-enrichment in multiple cancer-related KEGG pathways likely reflects shared signalling components rather than direct oncogenic roles, a common observation in pathway analyses of metabolic regulators.

Protein–protein interaction network analysis further demonstrated functional connectivity among these genes, with several acting as network hubs. Such centrality suggests that perturbations in these loci may have cascading effects across multiple biological systems.
By integrating GWAS association statistics, population genetics, genomic annotation, functional prediction, pathway enrichment, network topology, and structural modelling, this study provides a comprehensive framework for interpreting complex disease genetics. The findings demonstrate that **T2DM and CVD share overlapping molecular pathways**, particularly involving lipid metabolism, insulin signalling, inflammation, and vascular biology.
This systems-level integration enhances causal inference and facilitates the identification of high-priority variants for experimental validation and therapeutic targeting. Several findings, such as those involving **PCSK9 and PPARG**, have clear translational relevance, underscoring the value of integrative computational genomics in precision medicine.

CONCLUSION: This study aimed to elucidate the genetic architecture underlying Type 2 diabetes mellitus (T2DM) and cardiovascular disorders (CVD) through an integrative bioinformatics analysis of disease-associated single nucleotide polymorphisms (SNPs). Given the multifactorial nature and strong clinical interrelationship between these conditions, the study adopted a systems-level approach to move beyond

isolated genetic associations and uncover shared molecular mechanisms contributing to cardiometabolic disease risk.

A structured computational workflow was implemented, encompassing SNP retrieval from curated databases, rigorous filtering, genomic annotation, functional impact prediction, linkage disequilibrium analysis, and pathway and network-based interpretation. This approach enabled the translation of large-scale genomic data into biologically meaningful insights, addressing the gap between genome-wide association signals and mechanistic understanding.

The analysis identified multiple high-confidence SNPs associated with T2DM and CVD, mapping to genes with established roles in metabolic regulation, vascular biology, lipid metabolism, and inflammation. In T2DM, variants in *TCF7L2*, *PPARG*, *FTO*, *KCNJ11*, and *SLC30A8* were primarily linked to impaired pancreatic β-cell function, defective insulin secretion, adipogenesis, and insulin resistance. These findings reinforce the central role of dysregulated glucose homeostasis and energy balance in T2DM pathogenesis.

In CVD, SNPs in *APOE*, *PCSK9*, *NOS3*, and *IL6* were strongly associated with dyslipidaemia, endothelial dysfunction, reduced nitric oxide bioavailability, and chronic inflammatory signalling. These molecular processes are key drivers of atherosclerosis, hypertension, and coronary artery disease.

Importantly, integrative pathway enrichment and protein–protein interaction analyses revealed substantial overlap between T2DM- and CVD-associated genes. Shared pathways included insulin signalling, lipid metabolism, oxidative stress, endothelial dysfunction, and inflammatory cascades, providing genetic evidence for the close biological link between metabolic and cardiovascular disease.

A major contribution of this study is the demonstration of a shared genetic and molecular framework underlying T2DM and CVD. Inflammatory mediators such as *IL6* were shown to influence insulin resistance, β-cell stress, endothelial activation, and atherosclerotic plaque formation, highlighting inflammation as a central connecting mechanism. Similarly, lipid-related genes including *APOE* and *PCSK9* modulate both cholesterol homeostasis and metabolic risk, linking dyslipidaemia with glucose intolerance.

Endothelial dysfunction emerged as a critical mechanistic bridge between the two diseases. Variants in *NOS3* impair nitric oxide production, leading to vascular stiffness, reduced insulin-mediated glucose uptake, and increased cardiovascular risk. Hyperglycaemia-induced oxidative stress further amplifies vascular damage, establishing a feed-forward loop that accelerates cardiovascular complications in individuals with diabetes.

Collectively, these findings support the concept of a **cardiometabolic disease continuum**, in which T2DM and CVD represent interconnected manifestations of shared genetic, metabolic, and inflammatory disturbances rather than independent clinical entities. This study highlights the strength of integrative bioinformatics approaches in the investigation of complex diseases. The combined use of Ensembl Variant Effect Predictor (VEP), STRING, KEGG, and ShinyGO v0.85 enabled robust SNP prioritization, functional annotation, and systems-level interpretation.

By integrating variant-level predictions with pathway enrichment and network analyses, the study moved beyond single-gene interpretations to identify higher-order biological processes and regulatory hubs. The reproducible analytical framework presented here can be readily applied to other multifactorial disorders and contributes to translational genomics research by linking genetic variation to disease-relevant biological pathways. The identification of shared genetic pathways between T2DM and CVD has important biological and clinical implications. Genes such as *PPARG*, *KCNJ11*, *PCSK9*, and *APOE* represent potential therapeutic targets due to their central roles in cardiometabolic regulation and established pharmacological relevance.

From a clinical perspective, these findings support the integration of genetic data into cardiometabolic risk assessment. SNP-based insights may improve early disease prediction, enable more accurate risk stratification, and inform personalized prevention strategies. Furthermore, understanding genetic influences on drug response provides a foundation for pharmacogenomic-guided therapy aimed at optimizing treatment efficacy and reducing adverse outcomes.

In conclusion, this study demonstrates that integrative SNP-based bioinformatics analysis is a powerful approach for unraveling the complex genetic architecture of T2DM and CVD. By identifying shared and disease-specific genetic mechanisms, the study advances understanding of cardiometabolic disease biology and supports the development of personalized, mechanism-driven prevention and treatment strategies. Such integrative genetic insights are essential for addressing the growing global burden of diabetes and cardiovascular disease.

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