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Genetic and pathway analysis of ventricular septal defect: A comprehensive study

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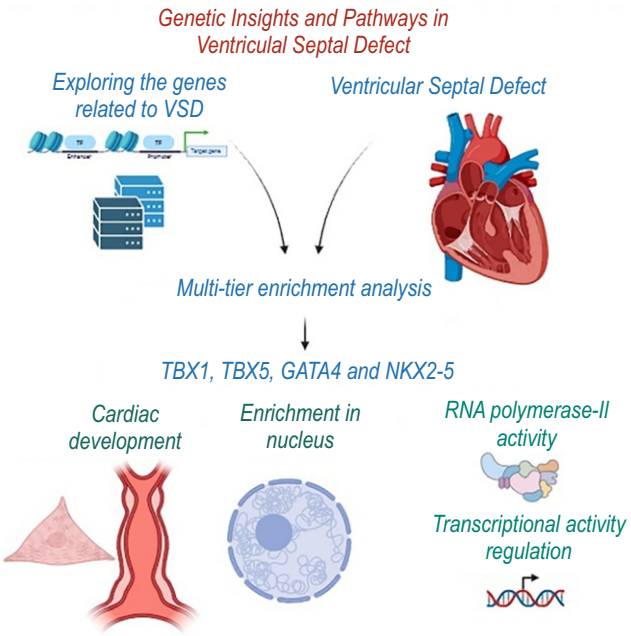
Abstract

Aim: Ventricular septal defect (VSD) is the most prevalent congenital cardiac malformation, yet its molecular and genetic determinants remain incompletely understood. This study aimed to elucidate the genetic contributors and biological pathways implicated in VSD pathogenesis.

Methodology: Thirty genes most strongly associated with VSD were retrieved from DisGeNET. Gene Ontology (GO) enrichment was performed across biological process, cellular component and molecular function categories. Pathway enrichment utilised WikiPathways, whilst metabolite and drug interaction profiling employed HMDB and DrugMatrix platforms. Tissue-specific and subcellular analyses were conducted via Jensen database resources, with all computations performed in R (v4.4.2).

Results: GO enrichment highlighted terms encompassing cardiac developmental biology, morphogenetic architecture and transcriptional regulation. WikiPathways analysis revealed significant enrichment for heart development ($p = 1.58 \times 10^{-15}$) and cardiac progenitor differentiation ($p = 1.46 \times 10^{-8}$), with *TBX1, TBX5, GATA4* and *NKX2-5* identified as central contributors. Tissue profiling confirmed pronounced cardiac-specific enrichment, and metabolite-drug screening identified associations with Simvastatin and Losartan.

Interpretation: These findings delineate the genetic landscape and molecular circuitry underlying VSD, underscoring critical roles of cardiac developmental genes and regulatory networks. Such insights may inform future therapeutic strategies and risk stratification for this common congenital anomaly.



Key words: Congenital heart disease, Cardiac development, Genetic pathways, Transcriptional regulation, Ventricular septal defect

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Introduction

Congenital cardiac malformations represent the most frequently occurring category of birth defects, with an estimated prevalence of 1% across all live births globally (Xu *et al.,* 2025). Within this spectrum, ventricular septal defect (VSD) accounts for the largest proportion, comprising approximately 30–40% of isolated congenital cardiac anomalies (Kahramanoglu *et al.,* 2024). VSD is characterised by an abnormal communication within the interventricular septum, permitting aberrant haemodynamic flow between left and right ventricular chambers. Clinical presentations vary considerably; smaller defects may remain asymptomatic with potential for spontaneous closure, whereas larger communications can precipitate significant haemodynamic compromise and progress towards cardiac failure (Meng *et al.,* 2024). The pathogenesis of VSD involves multifactorial contributions from both hereditary and environmental determinants. Whilst certain genetic aetiologies are well established in syndromic contexts—such as DiGeorge syndrome arising from TBX1 mutations—the majority of VSD cases lack clearly defined molecular origins (Cervantes-Salazar *et al.,* 2024).

Advances in genomic technologies have facilitated identification of multiple genes governing cardiac septation, a complex embryological process requiring coordinated cell proliferation, migration, and differentiation across multiple developmental stages (Lim *et al.,* 2021). Disruptions affecting these precisely orchestrated pathways can result in septal malformations. Key transcriptional regulators including *NKX2-5, GATA4,* and *TBX5* have been shown to play essential roles in septal morphogenesis (Bolunduț *et al.,* 2023). Contemporary genomic platforms and computational biology approaches enable researchers to examine disease mechanisms beyond isolated gene functions, permitting systematic exploration of broader genetic networks and pathway interactions. Gene Ontology (GO) frameworks provide structured annotation of biological processes, subcellular components, and molecular activities associated with disease-linked genes (Pitarch *et al.,* 2025). Complementary pathway analyses reveal functional networks and signalling cascades that may become perturbed in pathological states, potentially identifying intervention targets (Saravanan *et al.,* 2024). The DisGeNET resource serves as a comprehensive repository integrating gene-disease associations derived from literature curation, databases, and experimental animal models (Hu *et al.,* 2025).

Concurrently, investigations into metabolic dimensions of cardiac development and congenital disorders have gained momentum. The Human Metabolome Database (HMDB) catalogues small-molecule metabolites present in human biological systems, enabling examination of metabolic alterations associated with disease mechanisms (Wishart *et al.,* 2018). Such analyses may uncover biochemical pathway disturbances linked to cardiac developmental anomalies and genetic modifications in congenital heart conditions. Current VSD management relies predominantly on surgical repair or catheter-based interventions, with pharmacotherapy limited to symptomatic control rather than disease modification. Resources such as DrugMatrix, which catalogue drug-gene interactions, offer potential avenues for developing targeted therapies capable of modulating expression profiles of VSD-associated genes (Kim *et al.,* 2024).

Despite substantial progress in understanding VSD genetics, several challenges persist. The condition exhibits considerable genetic heterogeneity, variable expressivity, and incomplete penetrance, complicating genotype-phenotype correlations. Additionally, interactions between genetic susceptibility factors and environmental influences remain poorly characterised. Although numerous candidate genes have been implicated, mechanistic understanding of how specific alterations produce septal defects remains incomplete. This investigation aims to illuminate the genetic architecture and biological pathways underlying VSD through systematic examination of 30 genes demonstrating strongest disease associations in DisGeNET. Through comprehensive GO enrichment, pathway analysis, and assessment of metabolite-drug interactions, we seek to clarify molecular mechanisms driving VSD pathogenesis. By evaluating functional consequences of VSD-associated genes across diverse biological contexts, this work endeavours to connect genetic associations with functional understanding, potentially informing novel therapeutic and preventive strategies.

Materials and Methods

This investigation employed an integrative computational biology framework to examine genetic and molecular mechanisms underlying VSD. The top 30 VSD-associated genes were retrieved from DisGeNET based on gene-disease association scores, which aggregate evidence from literature mining, genome-wide association studies, animal models, and curated databases. All subsequent analyses were conducted using R version 4.4.2. Gene Ontology (GO) enrichment encompassed three domains: Biological Process, Cellular Component, and Molecular Function. Enrichment scores were computed as proportional representation within the gene set, with statistical significance assessed via Fisher's exact test followed by Benjamini-Hochberg adjustment for multiple comparisons. Results were visualised as bar plots displaying $-\log_{10}$ (adjusted p-value). Pathway enrichment utilised the WikiPaths_Human 2024 database to identify signalling networks overrepresented among VSD-associated genes. Overlap statistics, including p-values, odds ratios, and combined scores, were tabulated systematically. Subcellular distribution and tissue-specific expression were evaluated using Jensen_COMPARTMENTS and Jensen_TISSUES resources, respectively.

Transcription factor enrichment analysis via ChEA_2022 identified putative regulatory elements coordinating gene expression during cardiac development. Metabolite associations were examined through HMDB_Metabolites, whilst potential drug interactions were assessed using DrugMatrix, with enrichment statistics and contributing genes reported in tabular

format.Statistical significance was defined as adjusted $p < 0.05$ across all analyses. This multi-layered approach—integrating GO annotation, pathway mapping, localisation profiling, regulatory network analysis, and drug-metabolite interaction screening—provides a comprehensive view of the functional landscape associated with VSD-related genes and highlights potential avenues for further investigation.

Results and Discussion

Analysis of VSD-associated genes yielded substantial insight into genetic and molecular determinants of this prevalent congenital cardiac malformation. GO enrichment across biological processes revealed pronounced involvement of cardiac morphogenesis and heart developmental pathways (Fig. 1). The enrichment of cardiac morphogenesis and heart developmental pathways is not unexpected given the disease context, but the strength of the statistical signal here confirms that the top 30 VSD-associated genes retrieved from DisGeNET are genuinely concentrated around the biology of cardiac septation rather than being spread across unrelated functions.

This kind of focused enrichment in a disease-relevant biological process gives confidence that the gene set is capturing the core molecular players in VSD pathogenesis rather than background noise (Zhou *et al.,* 2023). These observations support a developmental aetiology for VSD, wherein disruptions to critical embryonic programmes may impair ventricular septation. Cellular component enrichment demonstrated that VSD-associated gene products localise preferentially to subcellular structures essential for normal cardiac morphogenesis, suggesting that architectural perturbations may contribute to septal malformations (Fig. 2). Disruption of precisely coordinated embryonic programmes during ventricular septation is a well-recognised mechanism in VSD and the GO biological process results here map directly onto this understanding. Genes like NKX2-5, GATA4, and TBX5 are expressed during critical windows of cardiac morphogenesis, and even heterozygous loss-of-function variants in these genes have been shown to produce septal defects in humans and animal models, demonstrating their dose-sensitive roles in this process (Ward *et al.,* 2025). Identification of these same genes as top contributors to the most significantly enriched GO terms in this analysis reinforces their biological centrality to VSD.

Molecular function enrichment similarly emphasised transcriptional regulation and signal transduction activities, implicating aberrant gene expression control and intercellular communication as central features of VSD pathogenesis (Fig. 3). Transcriptional regulation emerging as the dominant molecular function is consistent with what is known about VSD genetics, since the majority of well-characterised VSD-associated genes encode transcription factors rather than structural proteins. This reflects the fact that cardiac septation is fundamentally a transcriptionally driven process, where networks of transcription factors must be activated in precise temporal and spatial patterns to coordinate cell fate decisions in the developing heart (Li *et al.,* 2024). Aberrant gene expression control arising from variants in these transcription factors can therefore propagate developmental errors across entire programmes of cardiac morphogenesis, explaining how single gene mutations produce complex structural phenotypes like VSD.

WikiPathways analysis reinforced the developmental basis of VSD. The Heart Development pathway (*WP1591*) exhibited highly significant enrichment ($p=1.58\times10^{-15}$), encompassing eight genes (*TBX1, TBX20, GATA6, GATA4, NFATC1, NKX2-5, TBX5, ISL1*) with substantial odds ratios and combined scores, underscoring their critical importance in VSD. Cardiac Progenitor Differentiation (*WP2406*) also showed significant enrichment ($p = 1.46 \times 10^{-8}$), represented by five genes (*TBX20, GATA4, NKX2-5, TBX5, ISL1*) (Table 1). The extraordinarily high odds ratio of 201.35 for the Heart Development pathway reflects the fact that 8 out of 44 genes in

Table 1: Wiki Pathways enrichment results, displaying columns for Term, Overlap, P-value, Adjusted P-value, Old P-value, Odds Ratio, Combined Score, and Genes

Ontology	Overlap	P.value	Adjusted. P. value	Odds. Ratio	Combined. Score	Genes
Heart Development WP1591	8/44	0.000000000000001578537	0.000000000000235202	201.35354	6,862.58716	*TBX1;TBX20;GATA6; GATA4;NFATC1;NKX2-5; TBX5;ISL1*
Cardiac Progenitor Differentiation Wp2406	5/53	0.000000014591832875840	0.000001087091549250	83.00833	1,497.70308	*TBX20;GATA4; NKX2-5; TBX5;ISL1*
Extracellular Vesicles in The Crosstalk of Cardiac Cells Wp4300	2/19	0.000366077977298442014	0.018181872872489301	83.83613	663.36718	*GATA4;MMP9*
Hepatitis B Infection WP4666	3/151	0.001474899787777049975	0.048797105816886698	14.88138	97.01418	*NFATC1;BRAF;MMP9*
Bladder Cancer WP2828	2/40	0.001637486772378749997	0.048797105816886698	37.46617	240.33019	*BRAF;MMP9*

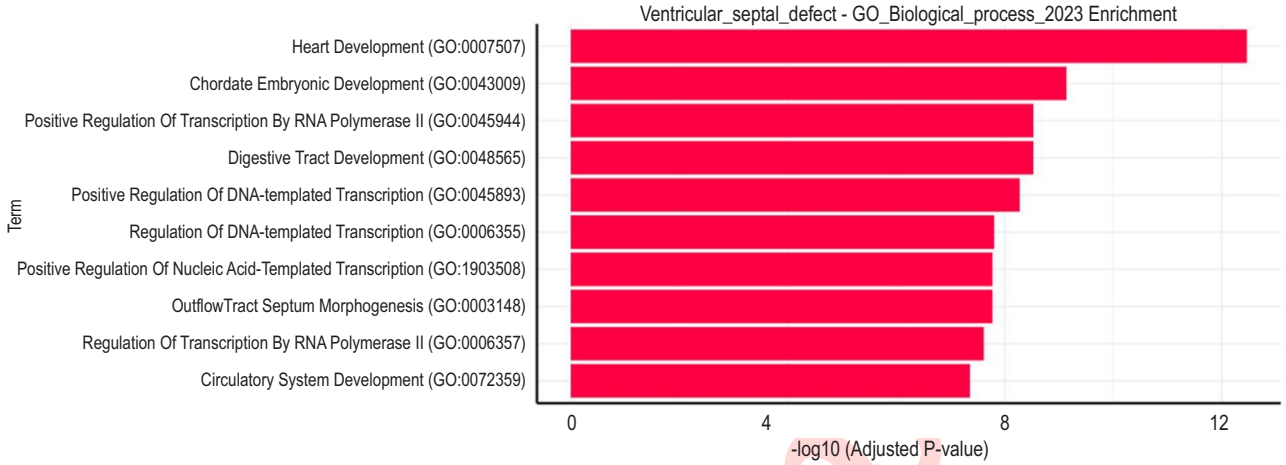

Fig. 1: Gene Ontology (GO) Biological Process 2023 Enrichment Analysis. Bar chart depicting enriched biological processes. The x-axis represents $-\log_{10}$ (adjusted p-value); y-axis displays corresponding GO terms. Only processes meeting adjusted $p < 0.05$ threshold were included.

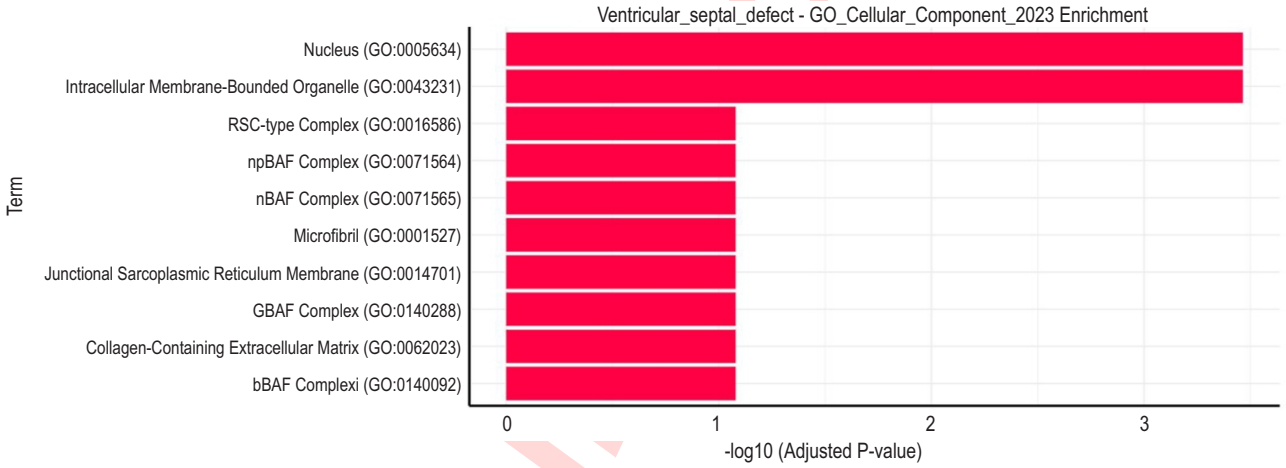

Fig. 2: Gene Ontology (GO) Cellular Component Analysis Results 2023. GO enrichment results displaying cellular components. Bars represent enriched compartments; x-axis shows $-\log_{10}$ (adjusted p-values); y-axis lists GO terms. Statistical significance threshold: adjusted p-value < 0.05.

this pathway were present in our VSD gene set, which is a very high hit rate and confirms genuine biological enrichment rather than coincidental overlap. The involvement of all three T-box family members—TBX1, TBX5 and TBX20 — alongside both GATA4 and GATA6, and the core cardiac transcription factors NKX2-5 and ISL1, suggests that the genetic architecture of VSD centres on the cooperative transcriptional network that these genes form during cardiac progenitor specification and differentiation (Stennard *et al.,* 2003). TBX20, for instance, directly interacts with NKX2-5 and GATA4 at shared target gene promoters, and disruption of any one node in this network is sufficient to impair the coordinated morphogenetic movements required for proper ventricular septation (Ward *et al.,* 2025). The enrichment of Cardiac Progenitor Differentiation as a second independent pathway, with an odds ratio of 83, further supports the conclusion that VSD originates at the level of progenitor cell programming rather than being a purely structural defect (Ameen *et al.,* 2022; Constantin *et al.,* 2022).

The Copy number variation syndromes demonstrated moderate enrichment, suggesting pleiotropic functions among VSD-related genes. Subcellular localisation analysis (Jensen_COMPARTMENTS) (Fig. 4) and tissue expression profiling (Jensen_TISSUES) confirmed predominant expression in cardiac tissue, though detection in other tissues indicated broader developmental roles. The pleiotropic functions suggested by CNV syndrome enrichment are clinically relevant because many patients with VSD present as part of broader syndromic conditions rather than as isolated cardiac defects, and this analysis provides a genetic explanation for why the same

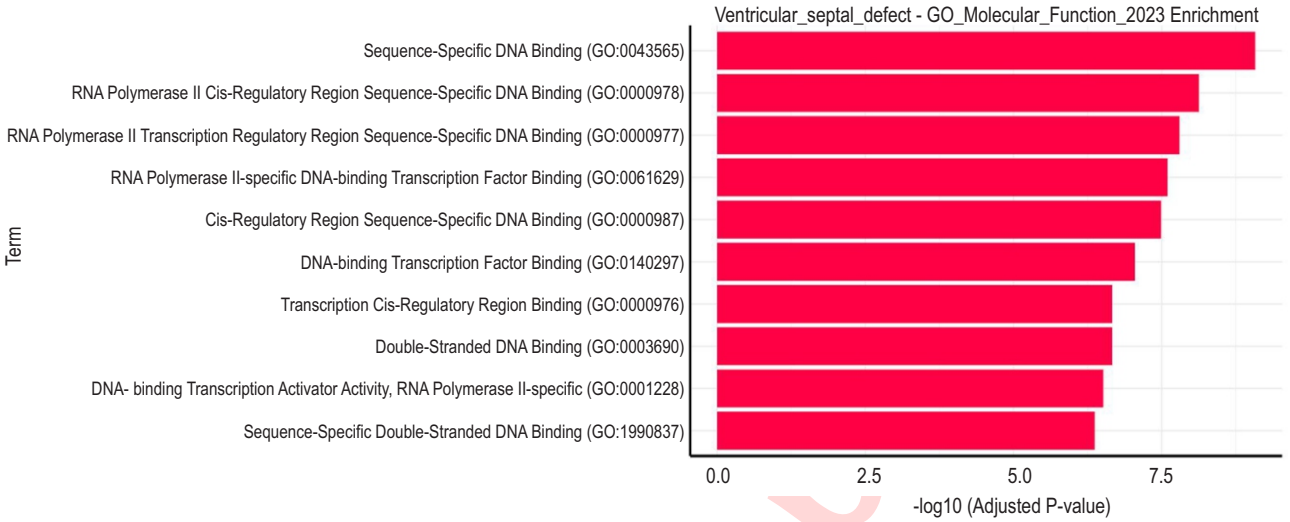

Fig. 3: Gene Ontology (GO) Molecular Function 2023 enrichment analysis. Bar chart illustrating significantly enriched molecular functions. x-axis: $-\log_{10}$ (adjusted p-values); y-axis: Gene Ontology terms. Selection criterion: adjusted p-value < 0.05.

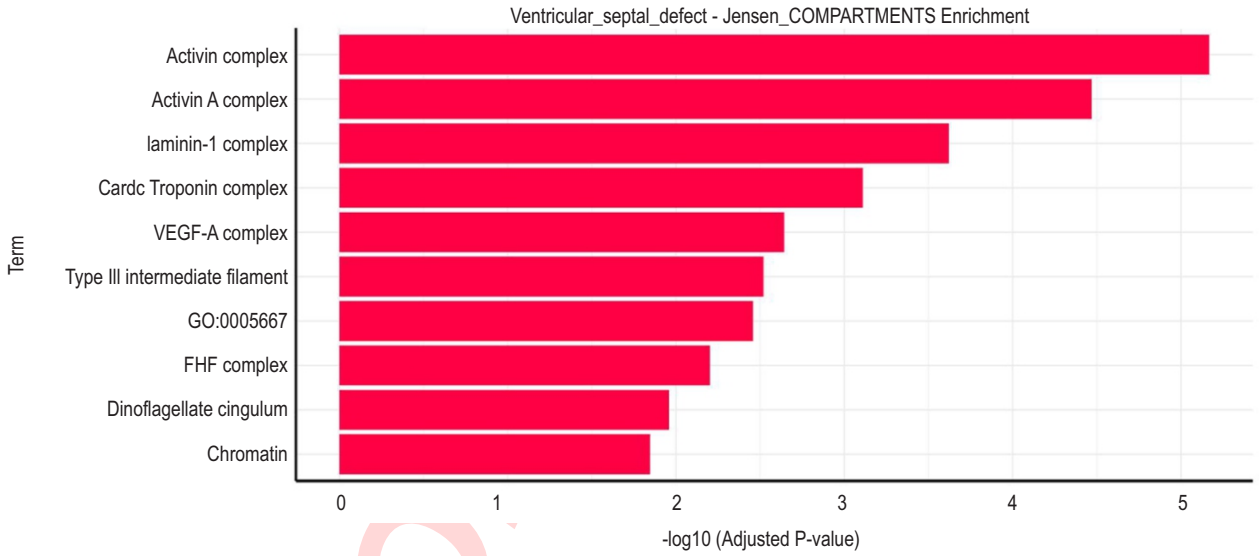

Fig. 4: Gjensen COMPARTMENTS enrichment analysis. Bar plot showing enriched cellular compartments; x-axis represents $-\log_{10}$ (adjusted p-values); y-axis lists compartment terms. Significance threshold: adjusted p < 0.05.

genes contribute to multiple phenotypic features (Chhatwal *et al.,* 2023). The cardiac-predominant tissue expression confirmed by Jensen_TISSUES is reassuring from a specificity standpoint, as it indicates that the identified genes are most highly expressed in the tissue most relevant to VSD, while their expression in other tissues accounts for the extra-cardiac features seen in syndromic cases (Hanson *et al.,* 2022). Transcription factor enrichment (ChEA_2022) identified key regulatory nodes that may orchestrate the coordinated expression of multiple VSD-associated genes, reinforcing the importance of transcriptional control in normal ventricular septation. Identification of master cardiac transcription factors as regulatory nodes through ChEA_2022 is consistent with the known hierarchical organisation of cardiac gene regulatory networks, where a small number of pioneer transcription factors occupy the top of the regulatory hierarchy and control the expression of hundreds of downstream target genes involved in septation, chamber formation, and valve development (Piven *et al.,* 2025).

Disruption of these master regulators through the

variants captured in VSD-associated loci would, therefore, be expected to produce cascading effects across multiple developmental programmes simultaneously. Transcription factor enrichment (ChEA_2022) identified key regulatory elements potentially orchestrating coordinated expression of VSD-associated genes.Metabolite and drug interaction analyses provided preliminary insights into molecular associations and pharmacological interactions. HMDB_Metabolites analysis revealed simvastatin as the compound with strongest correlation, followed by zinc and magnesium. DrugMatrix screening identified interaction potential for losartan and prednisolone. Whilst these associations did not achieve significance following multiple testing correction, they warrant further exploration.The association of simvastatin with VSD-related genes deserves attention in the clinical context, since statins are among the most widely prescribed medications and their use during early pregnancy, when cardiac septation is occurring, has raised safety concerns. The interaction identified here may reflect the influence of statin-sensitive pathways on the expression of genes like NKX2-5 or GATA4, which are critical during this developmental window (Hartley *et al.,* 2025). Losartan's interaction through TGF-beta signalling is also biologically plausible, since TGF-beta is a key regulator of cardiac mesenchymal transformation and endocardial cushion formation, the developmental process that precedes septal closure (Goumans and Ten Dijke, 2018).

Collectively, these findings delineate the genetic architecture of VSD, highlighting cardiac developmental programmes, transcriptional regulatory mechanisms, and potential interactions with metabolic compounds and pharmacological agents. This integrated framework not only emphasises the critical importance of precise molecular and developmental control, but also provides a foundation for investigating VSD pathophysiology and future therapeutic strategies. What this integrated analysis makes clear is that VSD is not caused by a single defective gene but by perturbations in a tightly interconnected transcriptional network that governs cardiac progenitor differentiation and septal morphogenesis. The fact that the same genes appear repeatedly across GO biological processes, WikiPathways, tissue expression, transcription factor, and metabolite analyses confirms that the signal is robust and biologically meaningful rather than platform-specific (Rosati *et al.,* 2024). This kind of cross-platform convergence is particularly important for a condition like VSD where the genetic heterogeneity is high and individual gene effects are often modest.

Our cross-platform gene analysis elucidates key molecular mechanisms underlying this prevalent congenital cardiac malformation. WikiPathways analysis demonstrated pronounced enrichment of heart development (WP1591) and cardiac progenitor differentiation (WP2406) pathways ($p = 1.58 \times 10^{-15}$; odds ratio=201.35), confirming the centrality of developmental gene networks in VSD pathogenesis (Sarwar *et al.,* 2022). Eight genes integral to cardiac morphogenesis constitute an essential regulatory circuit: *TBX1, TBX20, GATA6, GATA4, NFATC1, NKX2-5, TBX5* and *ISL1*. T-box transcription factors—*TBX1, TBX5* and *TBX20* were extensively implicated in congenital cardiac anomalies; *TBX1* haploinsufficiency underlies DiGeorge syndrome, whilst *TBX5* mutations cause Holt-Oram syndrome (Lozano-Velasco *et al.,* 2022). *GATA* family members, particularly *GATA4* functioning cooperatively with *NKX2-5* and *TBX5,* represent another well-characterised contributor to septal malformations (Abhinav *et al.,* 2024). The 8p23.1 copy-number variation syndrome, encompassing *GATA4* and *TBX5,* links both syndromic and isolated VSD presentations (Cicenia *et al.,* 2022). Enrichment extended beyond cardiac-specific pathways to include hepatitis B infection, bladder cancer, IL-26 signalling, and extracellular vesicle communication ($p = 0.00037$), indicating pleiotropic effects of developmental genes (Behiry *et al.,* 2019). *MMP9*, implicated in extracellular matrix remodelling during septation, was identified among these genes.

Metabolite and drug interaction analyses revealed modest associations with simvastatin ($p = 0.037$), relevant given concerns regarding statin exposure during pregnancy, and potential interactions between losartan and TGF-β signalling involving VSD-associated genes (Daud *et al.,* 2015). Gene expression extended beyond cardiac tissues, consistent with extra-cardiac manifestations observed in individuals carrying VSD-associated mutations (Henderson *et al.,* 2024). Clinically, identification of this transcriptional signature contributes to genetic risk stratification and may help explain variable expressivity (Xhonneux *et al.,* 2021). These pathways represent potential molecular targets for future gene-directed therapeutic approaches (Gaviglio *et al.,* 2023). Notwithstanding limitations inherent to gene and pathway selection criteria, these results advance understanding of molecular mechanisms underlying VSD and contribute meaningfully to existing knowledge. The core regulatory network identified extends current literature regarding VSD genetic architecture (Yasuhara and Garg, 2021).

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