Functional Pathway Analysis of Genetic Variants associated with Hypertension: A Comprehensive *In Silico* Approach

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Abstract

Hypertension is a complex multifactorial disorder with significant genetic components. Genome-wide association studies (GWAS) have identified several genetic variants associated with hypertension. This study aimed to identify biological pathways and molecular mechanisms potentially involved in hypertension pathogenesis through in silico functional analysis of GWAS-identified variants. We conducted a comprehensive pathway analysis of five genes (CHRM3, ATP2B1, CACNB2, MSRA, UMOD and ZNF831) previously associated with hypertension through GWAS. Multiple databases enriched biological processes, cellular components, molecular functions, metabolic pathways, miRNA targets, protein-protein interactions and gene expression signatures. Our analysis revealed significant enrichment in several pathways critical to hypertension pathophysiology. Key biological processes included regulation of smooth muscle contraction (p=2.65×10^-5), vasoconstriction (p=4.53×10^-5) and calcium ion transport (p=5.65×10^-5).

Molecular function analysis highlighted calcium ion transmembrane transporter activity (p=3.15×10^-4). Enriched cellular components included basolateral plasma membrane (p=1.97×10^-3) and voltage-gated calcium channel complexes (p=1.94×10^-2). Pathway analysis identified significant associations with sensory processing of sound (p=3.24×10^-4), cardiac conduction (p=1.14×10^-3) and regulation of insulin secretion (p=4.14×10^-4).

Our findings provide new insights into the molecular mechanisms underlying hypertension, particularly highlighting the importance of calcium signaling, smooth muscle contraction and vasoconstriction. These results suggest potential therapeutic targets and may contribute to the development of personalized medicine approaches for hypertension management.

Keywords: Hypertension, genome-wide association study, pathway analysis, calcium signaling, vasoconstriction.

Introduction

Hypertension is a complex, multifactorial disorder characterized by persistently elevated blood pressure, affecting approximately 1.28 billion adults worldwide.[20] As one of the leading risk factors for cardiovascular diseases, hypertension significantly contributes to global mortality and morbidity, accounting for an estimated 10.4 million deaths annually.[10]

Despite advancements in antihypertensive therapies, the understanding of the underlying pathophysiological mechanisms remains incomplete, hindering the development of more effective and personalized treatment approaches.[21]

The etiology of hypertension involves a complex interplay between genetic, environmental and lifestyle factors. The genetic component of hypertension has been estimated to account for approximately 30-50% of blood pressure variation, suggesting a substantial heritable contribution to the disorder.[24] Over the past decade, genome-wide association studies (GWAS) have emerged as powerful tools for identifying genetic loci associated with complex traits including hypertension.[9] These studies have successfully identified numerous single nucleotide polymorphisms (SNPs) associated with blood pressure regulation and hypertension risk.[5]

Among the genes identified through GWAS, several have shown consistent associations with hypertension across different populations. Notable examples include ATP2B1 (ATPase plasma membrane Ca^{2+} transporting 1), CACNB2 (calcium voltage-gated channel auxiliary subunit beta 2), CHRM3 (cholinergic receptor muscarinic 3), MSRA (methionine sulfoxide reductase A), UMOD (uromodulin) and ZNF831 (zinc finger protein 831).[32,36] These genes encode proteins involved in various biological processes including calcium homeostasis, smooth muscle contraction and renal function, all of which are relevant to blood pressure regulation.[22]

The ATP2B1 gene encodes a plasma membrane calcium ATPase responsible for calcium extrusion from cells, playing a crucial role in maintaining intracellular calcium homeostasis.[17] Variations in ATP2B1 have been consistently associated with hypertension risk across multiple ethnic groups, with particularly strong evidence from East Asian populations.[14] The CACNB2 gene encodes the β2 subunit of

voltage-dependent calcium channels which regulate calcium influx and subsequently influence vascular tone and cardiac contractility.[12] Polymorphisms in CACNB2 have been linked to alterations in blood pressure regulation and hypertension susceptibility.[3]

The CHRM3 gene encodes the M3 muscarinic acetylcholine receptor, which is expressed in vascular smooth muscle cells and plays a role in vasoconstriction and blood pressure regulation.[35] MSRA encodes methionine sulfoxide reductase A, an enzyme involved in protein repair and protection against oxidative stress, which is increasingly recognized as a contributing factor in hypertension pathogenesis.[28] The UMOD gene encodes uromodulin, a protein abundantly expressed in the kidney's thick ascending limb of the loop of Henle, where it influences sodium reabsorption and consequently affects blood pressure regulation.[33] Finally, ZNF831 encodes a zinc finger protein whose specific role in hypertension remains less characterized but has shown consistent association signals in GWAS studies.[29]

While GWAS has been instrumental in identifying genetic variants associated with hypertension, the functional implications of these variants and their integration into biological pathways remain incompletely understood.[2] The identified SNPs often lie in non-coding regions of the genome, complicating the interpretation of their biological significance.[19] Furthermore, the modest effect sizes of individual variants necessitate a more comprehensive approach in understanding their collective impact on disease pathogenesis.[34] Publicly available GWAS data on hypertension was analysed.

Objectives

1. To identify enriched biological pathways associated with hypertension-related genetic variants through comprehensive *in silico* analysis.
2. To elucidate the potential molecular mechanisms and functional implications of hypertension-associated genes identified through previous GWAS studies.
3. To explore the cellular components and molecular functions most significantly associated with hypertension-related genetic variants.
4. To identify potential therapeutic targets and biomarkers for hypertension based on pathway analysis of GWAS-identified genes.

Material and Methods

Study Design and Gene Selection: This study employed a comprehensive *in silico* approach to analyze the functional implications of genes previously associated with hypertension through genome-wide association studies (GWAS)[15].

We focused on six genes that have shown consistent associations with hypertension across multiple studies: CHRM3, ATP2B1, CACNB2, MSRA, UMOD and ZNF831. These genes were selected based on their statistical significance in previous GWAS meta-analyses and replication studies, as well as their potential biological relevance to blood pressure regulation mechanisms.

Data Sources and Pathway Analysis Tools: We utilized multiple bioinformatics databases and tools to conduct a comprehensive pathway analysis. The primary databases included Gene Ontology (GO) for biological processes, cellular components and molecular functions; Reactome and KEGG for pathway analysis; HMDB for metabolite associations; miRTarBase for miRNA target interactions. and protein-protein interaction (PPI) databases. Additionally, we employed RNAseq gene expression omnibus (GEO) signatures to identify gene expression patterns associated with our selected genes.

Gene Ontology (GO) Analysis: Gene Ontology analysis was performed to identify enriched biological processes, cellular components and molecular functions associated with the selected hypertension-related genes. We used the 2023 GO database, which provides a comprehensive and up-to-date resource for functional annotations. The analysis was conducted using the hypergeometric test to calculate the statistical significance of enrichment, with a p-value threshold of 0.05 considered significant. Adjusted p-values were calculated using the Benjamini-Hochberg method to control for multiple testing. The overlap between our gene set and GO terms was quantified and odds ratios were calculated to measure the strength of association.

Pathway Enrichment Analysis: To identify enriched biological pathways, we conducted pathway enrichment analyses using the KEGG 2021 Human database and Reactome Pathways 2024. The hypergeometric test was applied to calculate enrichment p-values, with adjusted p-values determined using the Benjamini-Hochberg method.

We considered pathways with adjusted p-values < 0.05 as significantly enriched. The overlap between our gene set and pathway gene sets was quantified and odds ratios were calculated to measure the strength of association.

Metabolite Association Analysis: To explore potential metabolite associations, we queried the Human Metabolome Database (HMDB). This analysis aimed to identify metabolites that might be affected by or interact with our selected hypertension-associated genes. Statistical significance was determined using the hypergeometric test with p-values adjusted for multiple testing using the Benjamini-Hochberg method.

miRNA Target Analysis: To investigate potential miRNA-mediated regulation of our selected genes, we conducted miRNA target analysis using the miRTarBase 2017 database. This database contains experimentally validated miRNA-target interactions. The hypergeometric test was applied to calculate enrichment p-values, with adjusted p-values determined using the Benjamini-Hochberg method.

We focused on miRNAs with significant associations (adjusted p-value < 0.05) to our gene set.

Protein-Protein Interaction (PPI) Network Analysis: To explore potential protein-protein interactions, we conducted PPI network analysis using established PPI databases. The analysis aimed to identify hub proteins that might interact with our selected genes, potentially forming functional networks relevant to hypertension pathophysiology. The hypergeometric test was applied to calculate enrichment p-values, with adjusted p-values determined using the Benjamini-Hochberg method.

Gene Expression Analysis: To investigate gene expression patterns associated with our selected genes, we analyzed RNAseq data from the Gene Expression Omnibus (GEO) database. We focused on expression signatures in which our genes were either up-regulated or down-regulated. The analysis aimed to identify potential functional contexts in which our genes might play a role in hypertension pathophysiology. Statistical significance was determined using the hypergeometric test, with p-values adjusted for multiple testing using the Benjamini-Hochberg method.

Statistical Analysis: For all analyses, statistical significance was determined using the hypergeometric test, which calculates the probability of observing a specific overlap between our gene set and the gene sets in the respective databases. P-values were adjusted for multiple testing using the Benjamini-Hochberg method to control the false discovery rate. We considered adjusted p-values < 0.05 as statistically significant. Odds ratios were calculated to measure the strength of association and combined scores were computed to integrate the significance level with the strength of association.

Data Integration and Interpretation: The results from the various analyses were integrated to provide a comprehensive understanding of the biological pathways and molecular mechanisms potentially involved in hypertension pathogenesis. We examined the overlap and convergence of findings across different analytical approaches to identify the most robust and biologically plausible mechanisms. The integrated findings were interpreted in the context of existing knowledge about hypertension pathophysiology and potential therapeutic targets.

Results

Table 1 explains the reported genes, mapped genes, chromosome numbers and positions of GWAS study results [R].

GO Biological Process Analysis: Analysis of the GO biological process database revealed significant enrichment of several processes critical to hypertension pathophysiology (Table 2). The most significantly enriched processes included regulation of smooth muscle contraction (p=2.65×10^-5, adjusted p=1.32×10^-3), regulation of vasoconstriction (p=4.53×10^-5, adjusted p=1.32×10^-3) and positive regulation of calcium ion transport (p=5.65×10^-5, adjusted p=1.32×10^-3). These processes are directly relevant to vascular tone regulation and blood pressure control.

The analysis identified CHRM3 and ATP2B1 as key genes involved in the regulation of smooth muscle contraction and vasoconstriction, with extremely high odds ratios of 369.89 and 277.33 respectively. These findings highlight the crucial role of these genes in mediating vascular smooth muscle contractility, which is directly related to blood pressure regulation.

CACNB2 and ATP2B1 were associated with positive regulation of calcium ion transport (odds ratio=246.48) and positive regulation of monoatomic ion transport (odds ratio=175.04), emphasizing their importance in calcium homeostasis. Calcium signaling is a fundamental mechanism in vascular smooth muscle contraction and cardiac function, both of which are central to blood pressure regulation.

Additionally, CHRM3 and MSRA were linked to macromolecule modification (odds ratio=84.02), while MSRA alone was associated with protein repair (odds ratio=713.86). These processes may contribute to hypertension pathophysiology through post-translational modifications of proteins involved in blood pressure regulation and protection against oxidative stress respectively.

Table 1
GWAS data

Region	CHR_ID	CHR_POS	Reported Gene (S)	Mapped Gene
15q26.2	15	96287321	intergenic	NR2F2-AS1
1q43	1	2.39E+08	CHRM3, ZP4, RYR2	MIPEPP2 - CHRM3
8p23.1	8	10214110	MSRA	MSRA
12q21.33	12	89615182	ATP2B1	ATP2B1
20q13.32	20	59183665	EDN3, ZNF831	ZNF831
10p12.31	10	18419869	CACNB2	CACNB2
16p12.3	16	20354332	UMOD	UMOD

Table 2
GO Biological Process 2023

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Regulation Of Smooth Muscle Contraction (GO:0006940)	2/20	2.650432	0.0013191270	0	0	369.8888	3897.96440	CHRM3;ATP2B1
Regulation Of Vasoconstriction (GO:0019229)	2/26	4.5282143	0.0013191270	0	0	277.3333333	2774.053787	CHRM3;ATP2B1
Positive Regulation Of Calcium Ion Transport (GO:0051928)	2/29	5.6534016	0.0013191270	0	0	246.48148148	2410.753549	CACNB2;ATP2B1
Positive Regulation Of Monoatomic Ion Transport (GO:0043270)	2/40	0.000108	0.001896544	0	0	175.0350877	1598.05683	CACNB2;ATP2B1
Regulation Of Calcium Ion Transport (GO:0051924)	2/57	0.000220	0.003093969	0	0	120.8303030	1017.07187	CACNB2;ATP2B1
Macromolecule Modification (GO:0043412)	2/81	0.000446	0.00520909	0	0	84.02109704	648.145836	CHRM3;MSRA
Protein Repair (GO:0030091)	1/5	0.00199	0.01566527	0	0	713.8571428	4436.85531	MSRA
Regulation Of Cellular Response To Insulin Stimulus (GO:1900076)	1/6	0.00239785	0.01566527417077	0	0	571.05714285 7142	3445.289982608	ATP2B1
AV Node Cell Action Potential (GO:0086016)	1/6	0.002397	0.0156 65274	0	0	571.0571428	3445.28998	CACNB2
Anterograde Trans-Synaptic Signaling (GO:0098916)	2/199	0.002651	0.0156 65274	0	0	33.49407783	198.707694	CHRM3;CACNB2

Table 3
GO Cellular Component 2023

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Basolateral Plasma Membrane (GO:0016323)	2/171	0.0019672	0.0216397	0	0	39.0986	243.62817388	UMOD;ATP2B1
Voltage-Gated Calcium Channel Complex (GO:0005891)	1/49	0.0194359	0.0856593	0	0	59.3571 42857	233.904 51356	CACNB2
Calcium Channel Complex (GO:0034704)	1/59	0.0233616	0.0856593	0	0	49.0985 22167	184.446 4409	CACNB2
Golgi Lumen (GO:0005796)	1/100	0.0393135	0.1081121	0	0	28.7056 27705	92.8967 8500	UMOD
Cilium (GO:0005929)	1/257	0.0983096	0.1889238	0	0	11.0133 92857	25.5470 3336	UMOD
Dendrite (GO:0030425)	1/27	0.103	0.188	0	0	10.474	23.803	CHRM3
Actin Cytoskeleton (GO:0015629)	1/327	0.1235748	0.1941890	0	0	8.61787 90534	18.0191 9439	MSRA
Neuron Projection (GO:0043005)	1/557	0.2022826	0.2639594	0	0	4.99383 35046	7.98059 2375	CHRM3
Cytoskeleton (GO:0005856)	1/599	0.2159668	0.2639594	0	0	4.63306 25895	7.10077 2140	MSRA
Intracellular Organelle Lumen (GO:0070013)	1/856	0.2953156	0.3248472	0	0	3.19749 37343	3.90001 6643	UMOD

ATP2B1 was also associated with regulation of cellular response to insulin stimulus (odds ratio=571.06), suggesting a potential link between hypertension and insulin signaling pathways, which is consistent with the known association between hypertension and insulin resistance. CACNB2 was linked to AV node cell action potential (odds ratio=571.06), highlighting its role in cardiac conduction, which can influence cardiac output and consequently affect blood pressure. Finally, both CHRM3 and CACNB2 were associated with anterograde trans-synaptic signaling (odds ratio=33.49), suggesting potential involvement in neural regulation of blood pressure.

GO Cellular Component Analysis: The GO cellular component analysis (Table 3) identified significant enrichment of several cellular components relevant to hypertension. The most significantly enriched component was the basolateral plasma membrane (p=1.97×10^-3, adjusted p=2.16×10^-2) which was associated with UMOD and ATP2B1 (odds ratio=39.10). This cellular component is particularly important for ion transport across epithelial cells, including those in the kidney, which plays a crucial role in sodium and water homeostasis and blood pressure regulation.

CACNB2 was associated with voltage-gated calcium channel complex (p=1.94×10^-2, adjusted p=8.57×10^-2) and calcium channel complex (p=2.34×10^-2, adjusted p=8.57×10^-2), with odds ratios of 59.36 and 49.10 respectively. These findings further emphasize the importance of calcium channels in the regulation of vascular tone and cardiac contractility, both of which are relevant to blood pressure control. UMOD was associated with Golgi lumen (p=3.93×10^-2, adjusted p=1.08×10^-1) and cilium (p=9.83×10^-2, adjusted p=1.89×10^-1), with odds ratios of 28.71 and 11.01 respectively. These cellular components are involved in protein processing and secretion, as well as sensing of extracellular signals, which may contribute to the role of UMOD in renal function and blood pressure regulation.

CHRM3 was associated with dendrite (p=1.03×10^-1, adjusted p=1.89×10^-1) and neuron projection (p=2.02×10^-1, adjusted p=2.64×10^-1), with odds ratios of 10.47 and 4.99 respectively. These findings suggest potential involvement of CHRM3 in neural regulation of blood pressure, possibly through cholinergic signaling in the autonomic nervous system.

MSRA was associated with actin cytoskeleton (p=1.24×10^-1, adjusted p=1.94×10^-1) and cytoskeleton (p=2.16×10^-1, adjusted p=2.64×10^-1), with odds ratios of 8.62 and 4.63 respectively. These cellular components are involved in cell shape and mechanical properties, which may be relevant to vascular smooth muscle cell function and vascular remodeling in hypertension.

Table 4
GO Molecular Function 2023

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Calcium Ion Transmembrane Transporter Activity (GO:0015085)	2/68	0.000314	0.005980	0	0	100.6363	811.5074	CACNB2; ATP2B1
Oxidoreductase Activity, Acting On A Sulfur Group Of Donors, Disulfide As Acceptor (GO:0016671)	1/6	0.00239785 89	0.01138983 01	0	0	571.057142 85	3445.28998	MSRA
G Protein-Coupled Acetylcholine Receptor Activity (GO:0016907)	1/6	0.002397	0.011389	0	0	571.0571	3445.289	CHRM3
G Protein-Coupled Neurotransmitter Receptor Activity (GO:0099528)	1/6	0.002397	0.011389	0	0	571.0571	3445.289	CHRM3
P-type Calcium Transporter Activity (GO:0005388)	1/8	0.003196	0.012144	0	0	407.8571	2343.484	ATP2B1
P-type Ion Transporter Activity (GO:0015662)	1/11	0.004392	0.013908	0	0	285.4571	1549.437	ATP2B1
Phosphatidylinositol Phospholipase C Activity (GO:0004435)	1/20	0.007973	0.015665	0	0	150.1729	725.5830	CHRM3
Acetylcholine Receptor Activity (GO:0015464)	1/20	0.007973	0.015665	0	0	150.1729	725.5830	CHRM3
G Protein-Coupled Serotonin Receptor Activity (GO:0004993)	1/21	0.008370	0.015665	0	0	142.6571	682.3340	CHRM3
High Voltage-Gated Calcium Channel Activity (GO:0008331)	1/23	0.009164	0.015665	0	0	129.6753	608.4900	CACNB2

GO Molecular Function Analysis: The GO Molecular Function analysis (Table 4) highlighted several molecular functions significantly enriched among the hypertension-associated genes. The most significantly enriched function was calcium ion transmembrane transporter activity (p=3.15×10^-4, adjusted p=5.98×10^-3), which was associated with CACNB2 and ATP2B1 (odds ratio=100.64). This function is directly relevant to calcium homeostasis, which plays a crucial role in vascular smooth muscle contraction and cardiac function.

MSRA was associated with oxidoreductase activity, acting on a sulfur group of donors, disulfide as acceptor (p=2.40×10^-3, adjusted p=1.14×10^-2), with an odds ratio of 571.06. This function is involved in protein repair and protection against oxidative stress, which is increasingly recognized as a contributing factor in hypertension pathogenesis.

CHRM3 was associated with G protein-coupled acetylcholine receptor activity (p=2.40×10^-3, adjusted p=1.14×10^-2), G protein-coupled neurotransmitter receptor activity (p=2.40×10^-3, adjusted p=1.14×10^-2), phosphatidylinositol phospholipase C activity (p=7.97×10^-3, adjusted p=1.57×10^-2), acetylcholine receptor activity (p=7.97×10^-3, adjusted p=1.57×10^-2) and G protein-coupled serotonin receptor activity (p=8.37×10^-3, adjusted p=1.57×10^-2), with odds ratios ranging from 142.66 to 571.06. These functions are involved in signal transduction pathways that can influence vascular tone and cardiac function, both of which are relevant to blood pressure regulation.

ATP2B1 was associated with P-type calcium transporter activity (p=3.20×10^-3, adjusted p=1.21×10^-2) and P-type ion transporter activity (p=4.39×10^-3, adjusted p=1.39×10^-2), with odds ratios of 407.86 and 285.46, respectively. These functions are directly involved in calcium extrusion from cells, crucial for maintaining intracellular calcium homeostasis and regulating vascular smooth muscle contraction. CACNB2 was associated with high voltage-gated calcium channel activity (p=9.16×10^-3, adjusted p=1.57×10^-2), with an odds ratio of 129.68. This function is involved in calcium influx into cells, which can influence vascular tone and cardiac contractility, both of which are relevant to blood pressure control.

HMDB Metabolites Analysis: The HMDB Metabolites analysis (Table 5) identified several metabolites potentially associated with the hypertension-related genes. CHRM3 was associated with 2-methyl-4-(4-methylpiperazin-1-yl)-10H-thieno[2,3-b][1,5]benzodiazepine (p=5.59×10^-3, adjusted p=1.44×10^-2), with an odds ratio of 219.55. This metabolite is related to the antipsychotic drug olanzapine, which has been associated with metabolic side effects including weight gain and hypertension. CACNB2 was associated with verapamil (p=7.18×10^-3, adjusted p=1.44×10^-2), with an odds ratio of 167.86. Verapamil is a calcium channel blocker used in the treatment of hypertension, highlighting the relevance of CACNB2 to calcium channel function and blood pressure regulation. ATP2B1 was associated with phosphate (p=1.24×10^-1, adjusted p=1.65×10^-1) and magnesium (p=1.71×10^-1, adjusted p=1.71×10^-1), with odds ratios of 8.59 and 6.04 respectively. These metabolites are involved in various physiological processes including ATP synthesis and enzymatic reactions, which may be relevant to the function of ATP2B1 in calcium transport and blood pressure regulation.

KEGG Pathway Analysis: The KEGG Pathway analysis (Table 6) revealed significant enrichment of several pathways relevant to hypertension. The most significantly enriched pathways included salivary secretion (p=5.88×10^-4, adjusted p=8.13×10^-3), pancreatic secretion (p=7.07×10^-4, adjusted p=8.13×10^-3) and adrenergic signaling in cardiomyocytes (p=1.52×10^-3, adjusted p=1.16×10^-2). These pathways involve CHRM3, ATP2B1 and CACNB2, with odds ratios ranging from 44.69 to 72.90. The calcium signaling pathway was also significantly enriched (p=3.83×10^-3, adjusted p=2.20×10^-2), involving CHRM3 and ATP2B1 (odds ratio=27.67). This pathway is directly relevant to vascular smooth muscle contraction and cardiac function, both of which are central to blood pressure regulation.

ATP2B1 was associated with endocrine and other factor-regulated calcium reabsorption (p=2.10×10^-2, adjusted p=6.33×10^-2) and mineral absorption (p=2.38×10^-2, adjusted p=6.33×10^-2), with odds ratios of 54.78 and 48.26 respectively. These pathways are involved in calcium homeostasis and renal function, which are important for blood pressure regulation. CHRM3 was associated with gastric acid secretion (p=3.00×10^-2, adjusted p=6.33×10^-2), insulin secretion (p=3.39×10^-2, adjusted p=6.33×10^-2) and taste transduction (p=3.39×10^-2, adjusted p=6.33×10^-2), with odds ratios ranging from 33.46 to 37.94. These pathways involve various physiological processes that may be indirectly related to blood pressure regulation through autonomic nervous system activity and metabolic effects.

CACNB2 was associated with arrhythmogenic right ventricular cardiomyopathy (p=3.04×10^-2, adjusted p=6.33×10^-2), with an odds ratio of 37.44. This pathway is related to cardiac function which can influence cardiac output and consequently affects blood pressure.

miRTarBase Analysis: The miRTarBase analysis (Table 7) identified several miRNAs potentially targeting the hypertension-associated genes. The most significantly enriched miRNA was hsa-miR-6865-3p (p=7.63×10^-4, adjusted p=1.20×10^-1), which was associated with CACNB2 and ZNF831 (odds ratio=63.74). This miRNA may play a role in regulating the expression of these genes, potentially influencing their function in blood pressure regulation.

Table 5
HMDB Metabolites

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
2-methyl-4-(4-methylpiperazin-1-yl)-10H-thieno[2,3-b][1,5]benzodiazepine (HMDB05012)	1/14	0.0055	0.014357	0	0	219.54	1138.8	CHRM3
Verapamil (HMDB01850)	1/18	0.007	0.014	0	0	167.8	828.65	CACNB2
Phosphate (HMDB01429)	1/32	0.123	0.165	0	0	8.591	17.938	ATP2B1
Magnesium (HMDB00547)	1/46	0.170	0.170	0	0	6.038	10.669	ATP2B1

Table 6
KEGG 2021 Human

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Salivary secretion	2/93	0.0005	0.0081	0	0	72.8	542.2	CHRM3;ATP2B1
Pancreatic secretion	2/102	0.000706866	0.008128961	0	0	66.30666	481.0	CHRM3;ATP2B1
Adrenergic signaling in cardiomyocytes	2/150	0.001518864	0.011644624	0	0	44.69369	290.0	CACNB2;ATP2B1
Calcium signaling pathway	2/240	0.003828383	0.022013206	0	0	27.66666	153.97	CHRM3;ATP2B1
Endocrine and other factor-regulated calcium reabsorption	1/53	0.0210078957	0.0633168434377	0	0	54.780219	211.60815	ATP2B1
Mineral absorption	1/60	0.0237	0.0633	0	0	48.2	180.5	ATP2B1
Gastric acid secretion	1/76	0.0300	0.0633	0	0	37.9	133.0	CHRM3
Arrhythmogenic right ventricular cardiomyopathy	1/77	0.030393210	0.063316844	0	0	37.436090	130.78433	CACNB2
Insulin secretion	1/86	0.0338	0.0633	0	0	33.45	113.2	CHRM3
Taste transduction	1/86	0.0338	0.0633	0	0	33.4	113.2	CHRM3

Other significantly enriched miRNAs included hsa-miR-4667-3p (p=4.11×10^-3, adjusted p=1.54×10^-1) and hsa-miR-5193 (p=5.35×10^-3, adjusted p=1.54×10^-1), both associated with CACNB2 and ZNF831, with odds ratios of 26.65 and 23.21 respectively.

ATP2B1 was associated with several murine miRNAs including mmu-miR-3967 (p=4.79×10^-3, adjusted p=1.54×10^-1), mmu-miR-5121 (p=5.19×10^-3, adjusted p=1.54×10^-1), mmu-miR-691 (p=9.16×10^-3, adjusted p=1.54×10^-1), mmu-miR-384-3p (p=9.56×10^-3, adjusted p=1.54×10^-1), mmu-miR-1196-5p (p=9.96×10^-3, adjusted p=1.54×10^-1) and mmu-miR-3074-5p (p=1.04×10^-2, adjusted p=1.54×10^-1), with odds ratios ranging from 114.10 to 259.49. These miRNAs may play roles in regulating ATP2B1 expression and function, potentially influencing calcium homeostasis and blood pressure regulation. CHRM3 was associated with hsa-miR-6766-3p (p=1.51×10^-2, adjusted p=1.54×10^-1), with an odds ratio of 77.05. This miRNA may be involved in regulating CHRM3 expression, potentially influencing cholinergic signaling and blood pressure regulation.

Protein-Protein Interaction Hub Analysis: The protein-protein Interaction Hub analysis (Table 8) identified several hub proteins potentially interacting with the hypertension-associated genes. The most significantly enriched hub protein was PRKACA (p=1.24×10^-2, adjusted p=1.61×10^-1), which was associated with CACNB2 and ATP2B1 (odds ratio=14.88). PRKACA encodes the catalytic subunit of protein kinase A, which is involved in various signaling pathways including those regulating vascular tone and cardiac function. Other hub proteins included ARF1 (p=5.66×10^-2, adjusted p=1.85×10^-1), ARF6 (p=6.34×10^-2, adjusted p=1.85×10^-1) and CALM3 (p=6.68×10^-2, adjusted p=1.85×10^-1), associated with CHRM3, CHRM3 and ATP2B1 respectively, with odds ratios ranging from 16.56 to 19.69. These proteins are

involved in various cellular processes including vesicle trafficking, signal transduction and calcium signaling, which may be relevant to blood pressure regulation.

MDM2 (p=7.61×10^-2, adjusted p=1.85×10^-1), ATM (p=8.54×10^-2, adjusted p=1.85×10^-1), PRKDC (p=1.12×10^-1, adjusted p=1.95×10^-1) and PRKCB (p=1.27×10^-1, adjusted p=1.95×10^-1) were associated with CHRM3, CACNB2, CACNB2 and CACNB2, respectively, with odds ratios ranging from 8.33 to 14.43. These proteins are involved in various cellular processes including DNA damage response, cell cycle regulation and signal transduction which may be indirectly related to blood pressure regulation. CALM1 (p=1.35×10^-1, adjusted p=1.95×10^-1) and DLG4 (p=1.52×10^-1, adjusted p=1.98×10^-1) were associated with ATP2B1, with odds ratios of 7.83 and 6.86 respectively. These proteins are involved in calcium signaling and synaptic function, which may be relevant to neural regulation of blood pressure.

Reactome Pathway Analysis: The Reactome pathway analysis (Table 9) revealed significant enrichment of several pathways relevant to hypertension. The most significantly enriched pathways included sensory processing of sound by inner hair cells of the cochlea (p=3.24×10^-4, adjusted p=6.35×10^-3), sensory processing of sound (p=4.04×10^-4, adjusted p=6.35×10^-3) and regulation of insulin secretion (p=4.14×10^-4, adjusted p=6.35×10^-3). These pathways involve CACNB2, ATP2B1 and CHRM3, with odds ratios ranging from 87.35 to 99.13.

Other significantly enriched pathways included integration of energy metabolism (p=7.92×10^-4, adjusted p=9.11×10^-3), cardiac conduction (p=1.14×10^-3, adjusted p=1.05×10^-2) and muscle contraction (p=2.78×10^-3, adjusted p=1.60×10^-2), involving CHRM3, CACNB2 and ATP2B1, with odds ratios ranging from 32.66 to 62.53. These pathways are directly relevant to cardiovascular function and blood pressure regulation.

Table 7
miRTarBase 2017

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
hsa-miR-6865-3p	2/106	0.000763	0.11980	0	0	63.74	457.5	CACNB2;ZNF831
hsa-miR-4667-3p	2/249	0.004114	0.15377	0	0	26.64	146.3	CACNB2;ZNF831
mmu-miR-3967	1/12	0.004790	0.15377	0	0	259.4	1385.9	ATP2B1
mmu-miR-5121	1/13	0.005189	0.15377	0	0	237.8	1251.4	ATP2B1
hsa-miR-5193	2/285	0.00535	0.15377	0	0	23.21	121.41	CACNB2;ZNF831
mmu-miR-691	1/23	0.009164	0.15377	0	0	129.6	608.49	ATP2B1
mmu-miR-384-3p	1/24	0.009561	0.15377	0	0	124.0	576.74	ATP2B1
mmu-miR-1196-5p	1/25	0.009957	0.15377	0	0	118.8	547.85	ATP2B1
mmu-miR-3074-5p	1/26	0.010354	0.15377	0	0	114.0	521.46	ATP2B1
hsa-miR-6766-3p	1/38	0.015101	0.15377	0	0	77.04	323.05	CHRM3

Table 8
PPI Hub Proteins

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
PRKACA	2/440	0.0123	0.1609	0	0	14.881	65.348	CACNB2;ATP2B1
ARF1	1/145	0.0565	0.1851	0	0	19.690	56.560	CHRM3
ARF6	1/163	0.0633	0.1851	0	0	17.486	48.238	CHRM3
CALM3	1/172	0.0667	0.1851	0	0	16.558	44.815	ATP2B1
MDM2	1/197	0.0761	0.1851	0	0	14.428	37.154	CHRM3
ATM	1/222	0.0854	0.1851	0	0	12.780	31.438	CACNB2
PRKDC	1/296	0.1124	0.1948	0	0	9.5384	20.842	CACNB2
PRKCB	1/338	0.1274	0.1948	0	0	8.3319	17.161	CACNB2
CALM1	1/359	0.1349	0.1948	0	0	7.8347	15.693	ATP2B1
DLG4	1/409	0.1523	0.1980	0	0	6.8571	12.900	ATP2B1

Table 9
Reactome Pathways 2024

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Sensory Processing of Sound by Inner Hair Cells of the Cochlea	2/69	0.000	0.0063	0	0	99.12	796.4	CACNB2;ATP2B1
Sensory Processing of Sound	2/77	0.0004	0.0063	0	0	88.52	691.8	CACNB2;ATP2B1
Regulation of Insulin Secretion	2/78	0.0004	0.0063	0	0	87.35	680.4	CHRM3;CACNB2
Integration of Energy Metabolism	2/108	0.0007	0.0091	0	0	62.53	446.5	CHRM3;CACNB2
Cardiac Conduction	2/130	0.0011	0.0105	0	0	51.72	350.3	CACNB2;ATP2B1
Muscarinic Acetylcholine Receptors	1/5	0.0019	0.0153	0	0	713.8	4436.	CHRM3
Protein Repair	1/6	0.0023	0.0157	0	0	571.0	3445.2	MSRA
Muscle Contraction	2/204	0.0027	0.0160	0	0	32.65	192.1	CACNB2;ATP2B1
Acetylcholine Regulates Insulin Secretion	1/10	0.0039	0.0198	0	0	317.1	1751.8	CHRM3
Presynaptic Depolarization and Calcium Channel Opening	1/12	0.004790	0.0198 91252	0	0	259.4 9350	1385.9750 83	CACNB2

Table 10
RNAseq GEO Signatures Human Down

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Hsa-Mir-503 Hsa-Mir-103 Hsa-Mir-494 Rip-Seq GSE64615 1	2/250	0.0041464 3179513	0.0618015786 6079	0	0	26.537 634408 6021	145.57 238257 5649	CHRM3;CACNB2
Integrator Subunit 12 Synthesis GSE80386 1	2/250	0.0041464 31	0.061801578	0	0	26.537 63440	145.57 23825	MSRA;ATP2B1
Integrator Subunit 12 Synthesis GSE80386 2	2/250	0.0041464 31	0.061801578	0	0	26.537 63440	145.57 23825	MSRA;ATP2B1
Integrator Subunit 12 Synthesis GSE80386 3	2/250	0.0041464 31	0.061801578	0	0	26.537 63440	145.57 23825	MSRA;ATP2B1
Roll Shear Tether Sling GSE107981 1	2/250	0.0041464 31	0.061801578	0	0	26.537 63440	145.57 23825	ATP2B1;ZNF831
Neutrophils Infected Iav Using GSE108807 1	2/250	0.0041464 31	0.061801578	0	0	26.537 63440	145.57 23825	ATP2B1;ZNF831
Network Androgen Receptor Epididymis GSE109062 1	2/250	0.0041464 31	0.061801578	0	0	26.537 63440	145.57 23825	CHRM3;CACNB2
Sox2-Dependent Circuit Drives Glioblastoma GSE58345 1	2/250	0.0041464 31	0.061801578	0	0	26.537 63440	145.57 23825	MSRA;CACNB2
Diarrhea 2-Dependent Claudin-4 8-Related GSE65107 1	2/250	0.0041464 31	0.06180157	0	0	26.537 63440	145.57 23825	CACNB2;ATP2B1
Il3R?-High Precursors M? Oc GSE65996 1	2/250	0.0041464 31	0.061801578	0	0	26.537 63440	145.57 23825	CHRM3;CACNB2

CHRM3 was associated with muscarinic acetylcholine receptors (p=2.00×10^-3, adjusted p=1.53×10^-2) and acetylcholine regulates insulin secretion (p=3.99×10^-3, adjusted p=1.99×10^-2), with odds ratios of 713.86 and 317.19 respectively. These pathways highlight the role of cholinergic signaling in metabolic regulation, which may be indirectly related to blood pressure regulation.

MSRA was associated with protein repair (p=2.40×10^-3, adjusted p=1.58×10^-2), with an odds ratio of 571.06. This pathway is involved in protection against oxidative stress which is increasingly recognized as a contributing factor in hypertension pathogenesis. CACNB2 was associated with presynaptic depolarization and calcium channel opening (p=4.79×10^-3, adjusted p=1.99×10^-2), with an odds ratio of 259.49. This pathway is directly relevant to synaptic transmission and neural regulation of blood pressure.

The RNA-seq results from the GEO database reveal interesting patterns potentially relevant to hypertension mechanisms. Looking across both tables of up-regulated and down-regulated signatures, several genes emerge as key players, with ATP2B1 appearing most frequently throughout the analysis. This gene encodes a plasma membrane calcium-transporting ATPase that plays a critical role in calcium homeostasis, a process fundamentally linked to vascular tone regulation and blood pressure control. The consistent appearance of CACNB2, which encodes a voltage-dependent calcium channel subunit, further strengthens the calcium signaling connection to hypertension. This aligns with established knowledge that calcium dynamics are central to vascular smooth muscle contraction and blood pressure regulation. CHRM3, another recurring gene in these signatures, encodes a muscarinic acetylcholine receptor involved in smooth muscle contraction and has been implicated in vascular function, providing another potential link to blood pressure regulation.

The statistical metrics across these signatures: identical p-values of 0.0041, odds ratios of 26.54 and combined scores of 145.57, suggest that these are not random associations but potentially meaningful biological connections. The adjusted p-values (0.062 for down-regulated and 0.096 for up-regulated) indicate these findings approach but do not quietly reach the conventional significance threshold of 0.05, suggesting interpretative caution while still acknowledging their potential biological relevance.

Discussion

Pathway analysis offers a valuable approach in bridging the gap between genetic associations and biological mechanisms by aggregating the effects of multiple variants within functional pathways[23].

Table 11
RNAseq GEO Signatures Human Up

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Salivary Duct New Opportunities GSE138581 1	2/250	0.00414 6431	0.095748	0	0	26.537 63440	145.57238 25	CHRM3;CACNB2
Screening Predicts Tyrosine Cardiotoxicity GSE88944 1	2/250	0.00414 6431	0.095748	0	0	26.537 63440	145.57238 25	CACNB2;ATP2B1
Comparing Omental Ovarian Samples GSE98281 1	2/250	0.00414 6431	0.095748	0	0	26.537 63440	145.57238 25756	MSRA;ZNF831
Leukaemic Ikzf1 Primes Malignancy GSE112124 2	2/250	0.00414 6431	0.095748	0	0	26.537 63440	145.57238 25756	ATP2B1;ZNF831
Kmt2A Rearranged Molm14 Dot1L GSE149520 2	2/250	0.00414 6431	0.095748	0	0	26.537	145.572	MSRA;ATP2B1
Clonal Replacement Tumor-Specific Pd-1 GSE123812 1	2/250	0.00414 6431	0.095748	0	0	26.537 63440	145.57238 25756	ATP2B1;ZNF831
Clonal Replacement Tumor-Specific Pd-1 GSE123812 2	2/250	0.00414 6431	0.095748	0	0	26.537 63440	145.57238 25756	ATP2B1;ZNF831
Clonal Replacement Tumor-Specific Pd-1 GSE123812 3	2/250	0.00414 6431	0.095748	0	0	26.537 63440	145.57238 25756	ATP2B1;ZNF831
Splice Variants Preferred Bindings GSE80741 1	2/250	0.00414 6431	0.095748	0	0	26.537 63440	145.572	CHRM3;ATP2B1
Kdm2B Leads Deregulation Related GSE81043 1	1/250	0.09574 8399	0.095748	0	0	11.327 02237	26.573549 77580	ATP2B1

This approach can provide insights into the molecular processes underlying hypertension and potentially identify novel therapeutic targets. By integrating GWAS findings with functional annotations from various databases, pathway analysis can help to elucidate the biological context of genetic associations and reveal enriched pathways relevant to disease pathophysiology[30]. Several pathway analysis approaches have been developed, including gene set enrichment analysis (GSEA), gene ontology (GO) analysis and protein-protein interaction (PPI) network analysis[7]. These methods allow for the identification of biological processes, molecular functions and cellular components that are significantly enriched among genes associated with hypertension[31]. Additionally, analyses of metabolic pathways, miRNA targets and gene expression signatures can provide further insights into the regulatory mechanisms underlying hypertension[13].

Previous studies have applied pathway analysis to GWAS data for hypertension, identifying enriched pathways related to calcium signaling, renin-angiotensin system and vascular smooth muscle contraction[11].For instance, a study by Ehret et al[6] identified enrichment in pathways related to calcium and sodium handling, as well as cardiovascular development, among hypertension-associated genes. Similarly, Evangelou et al[8] reported enrichment in pathways involved in vascular smooth muscle contraction and cardiac muscle contraction. These findings highlight the potential of pathway analysis to identify biologically relevant processes involved in hypertension pathogenesis.

Despite these advances, a comprehensive integration of multiple pathway analysis approaches applied to the most consistently replicated hypertension-associated genes remains limited. Such an integrative approach could provide a more holistic understanding of the biological mechanisms underlying hypertension and potentially identify novel therapeutic targets[27]. By combining analyses of biological processes, cellular components, molecular functions, metabolic pathways, miRNA targets, protein-protein interactions and gene expression signatures, a more comprehensive picture of the molecular landscape of hypertension can be constructed[1].In this study, we conducted a comprehensive pathway analysis of five genes (CHRM3, ATP2B1, CACNB2, MSRA, UMOD and ZNF831) previously associated with hypertension through GWAS.

We employed multiple databases and analysis tools to identify enriched biological pathways and molecular mechanisms potentially involved in hypertension pathogenesis. This approach allowed us to gain insights into the functional implications of these genetic associations and identify potential therapeutic targets for hypertension[16]. By integrating these diverse analyses, we aimed to provide a comprehensive understanding of the molecular mechanisms underlying hypertension and to identify novel avenues for therapeutic intervention[4]. The identification of enriched pathways can guide the development of targeted therapies that address the specific molecular mechanisms underlying an individual's hypertension[18]. Furthermore, the integration of pathway analysis with other omics data, such as transcriptomics and proteomics, can provide a more comprehensive understanding of the complex interplay between genetic variants and environmental factors in hypertension pathogenesis[25].

In conclusion, this study represents a comprehensive effort to elucidate the biological pathways and molecular mechanisms underlying hypertension through *in silico* analysis of GWAS-identified genes.

Our findings may contribute to the development of more effective and personalized approaches to hypertension management, ultimately improving patient outcomes and reducing the global burden of hypertension-related diseases[26]. The presence of these genes across diverse experimental contexts, from androgen receptor networks to studies of tyrosine kinase cardiotoxicity, hints at potential intersections between hypertension mechanisms and other physiological pathways.

In summary, these RNA-seq results point toward altered calcium signaling and ion transport as potential molecular mechanisms underlying hypertension, with ATP2B1 and CACNB2 emerging as particularly interesting candidates. These findings align with genome-wide association studies that have previously linked variants in these genes to hypertension risk, suggesting that they may represent valuable targets for further investigation and potential therapeutic development.

Limitations

It is important to acknowledge the limitations of our *in silico* approach. The analyses are based on existing databases and annotations, which may not be complete or may contain biases. Additionally, the functional implications of genetic variants may not be fully captured by pathway analysis alone, as they may involve complex gene-gene and gene-environment interactions.

Conclusion

The findings from this comprehensive analysis highlight the significant involvement of calcium ion regulation, cellular signaling and metabolic pathways in hypertension pathogenesis. The consistent identification of genes such as ATP2B1, CACNB2 and CHRM3 across various analyses reinforces their crucial roles in hypertension. Additionally, metabolite and miRNA interactions identified suggest potential novel biomarkers and therapeutic targets.

Collectively, these insights advance the understanding of hypertension at the molecular and cellular levels, offering promising avenues for targeted therapeutic strategies and personalized medicine approaches in managing hypertension effectively.

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