**Network Pharmacological analysis of primary targets underlying Pulmonary Hypertension**

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**Abstract**

The emerging subject of network pharmacology uses networks of disease-gene-drug targets to better understand the bimolecular level pharmacological mechanism of active components, which in turn aids in drug discovery. When blood pressure rises in the pulmonary arteries, it's called hypertension. Appearing when pulmonary arterial pressure is extremely high. Globally, pulmonary hypertension is thought to affect about 1% of the population. Using a network pharmacological strategy based on a string database, we identified particular genes associated in pulmonary hypertension, extracted gene ontology, biological pathways, and performed a protein-protein interaction analysis on the screened 100 proteins and 1910 PPI connections. The top 10 targets with the highest degree ratings in CytoScape\_v3.9.1 could be considered central targets. This chapter focused on the top 5 gene targets associated with hypertension, including EDN1, VEGFA, ALB, AKT1, and TNF. With this approach, we found that most of AKT1's protein-protein interactions are similar to those of other genes. Our data implies that the AKT1 gene, via several pathways, may be a novel therapy option for pulmonary hypertension; however, more preclinical and clinical research is needed to verify our findings.

**Keywords**: AKT1, Network pharmacology, Cytoscape, Target, Hypertension

**Introduction**

Abnormally high pressures in the pulmonary arteries characterize a set of diseases known collectively as *pulmonary hypertension*. Understanding the root of the problem is essential for developing a therapeutic strategy. Treatment for pulmonary hypertension typically focuses on the underlying condition, which is common in the advanced stages of chronic obstructive pulmonary disease (COPD) and left heart disease. Right-sided cardiac catheterization confirms the diagnosis of pulmonary hypertension (PH) if the resting mean pulmonary arterial pressure (mPAP) is 20 mm Hg or above(1). Pulmonary hypertension affects approximately 1% of the global population, up to 10% of individuals older than 65 years, and at least 50% of patients with heart failure (HF).2 Cardiologists can therefore expect to encounter PH in their clinical practice. This article provides an overview of the diagnosis and management of PH(2). Primary vasculopathies and chronic organised thromboembolism are less common causes of pulmonary hypertension. Both can be treated with cutting-edge medical therapy, although the former requires examination for surgical surgery(3). Patients with pulmonary hypertension (PH) may fall into one of five clinical subgroups: those with pulmonary arterial hypertension (PAH), those with PH caused by left-sided heart disease, those with PH caused by chronic lung illness, those with PH caused by chronic thromboembolic mechanisms, and those with PH caused by unclear and/or multifactorial mechanisms. These problems can develop from a wide variety of causes. About one percent of people worldwide have PH, and it's possible that more than half of those with heart failure have it. Therefore, PH is a common condition seen by cardiologists. Electrocardiography, chest radiography, and pulmonary function testing are the usual diagnostic procedures for individuals with symptoms and physical findings consistent with PH. To calculate a rough likelihood of PH, transthoracic echocardiography is employed. To rule out CTEPH, a ventilation-perfusion scan should be performed on all patients with suspected or proven PH who do not have left-sided heart or lung problems. For proper diagnosis and categorization, a right cardiac catheterization is required. It is mandatory to refer all patients with PAH or CTEPH to a specialised facility. CTEPH patients who are surgical candidates typically undergo pulmonary endarterectomy. Patients with PAH have access to a wide variety of approved therapies, including specific inhibitors of phosphodiesterase type 5, stimulators of soluble guanylate cyclase, antagonists of endothelin receptors, analogues of prostacyclin, and agonists of the prostacyclin receptor. Treatment of the underlying problem is the primary focus of management of PH due to left-sided cardiac disease(4) by identifying the specific target responsible for PH through network pharmacology.

**Network pharmacology**

Network pharmacology is a computational artificial intelligence (AI) technique developed in the 20th century, reduces the amount of time it takes to identify a potential drug discovery or protein/gene target for specific disease. It will predict the mechanism of drug functions in disease therapy as a whole is consistent with the holistic perspective of traditional medicine and is analogous to the mechanism of multiconstituents and multitargeting function of traditional medicine compounds (5-7). Network pharmacology is an emerging science based on the network of disease-gene-drug targets, which can provide a deep insight or scientific proof for the drug discovery and allow us to elucidate the pharmacological mechanism of active components at the biomolecule level. It is rapidly becoming a holistic and efficient tool for describing the complex interactions between medications and biological systems such as human organs, diseases, metabolic pathways, and target proteins (8). The Network pharmacology paradigm, which combines computational prediction and experimental validation, offers a fresh approach to investigating the mechanism of action of individual compounds (6). Synergistic multi-compound network pharmacology and drug repurposing provide precise and effective therapeutic intervention, eliminating the requirement for drug discovery and speeding up clinical translation (9). Combining a Network pharmacological approach with herbal medicine could help pinpoint the active natural biomolecule in instantaneously.

*Target Screening and Indications for Pulmonary hypertension Management*

Searches for "Pulmonary hypertension" in string database (<https://string-db.org/>) obtained list of genes were merged and deduplicated. After normalising the data on the UniProt database, we were able to gain the pertinent targets of pulmonary hypertension treatment. 100 identical targets were discovered from string database and further protein-protein interaction analysis was performed.

*Constructing of Protein-Protein Interaction (PPI) Network*

Analysis of Protein-Protein Interaction (PPI) is crucial for dissecting complex cellular mechanisms and gaining insight into biological processes. The STRING tool was used to build the PPI network. To obtain the PPI network relationship, the aforementioned intersecting targets were entered into STRING, the species was set to "homo sapiens," and the interaction score was set to 0.4. CytoScape\_v3.9.1 was used to create the PPI network diagram. The network analyzer feature was used to determine the accuracy of the value. Central targets could be defined as the top 10 targets with the highest degree scores. In order to learn more about PPI networks, the STRING database was populated with data from 100 intersection targets. There are 100 proteins represented as nodes in the PPI network and 1910 PPI relationships represented as edges were illustrated in [Fig.1](https://outlook.live.com/owa/wopi/files/00037ffe-6235-b97b-0000-000000000000%40outlook.com/AQMkADAwATM3ZmYAZS02MjM1LWI5N2ItMDACLTAwCgBGAAADcPfeAgTeMUy1Nayt2cyZ4QcAqt5JV48GKEO1Xa1n2ARqyAAAAgEMAAAAqt5JV48GKEO1Xa1n2ARqyAAAAErtfGkAAAABEgAQAMNRcwfM90FJkMj1unMEtsQ%3D_AADbmXo.MQkAAAAAAAA%3D/WOPIServiceId_FP_EXCHANGE_MSA/WOPIUserId_5187E4224B4168DD/Fig.2). Cytoscape v\_3.9.1 was used to visualise the information on protein interactions in the PPI network. After collecting the PPI network, we used the network analyzer to determine the degree value. EDN1, VEGFA, ALB, AKT1, TNF, NOS3, IL6, INS, IL1B, and ACTB were the top 10 targets according to degree value, with 77, 76, 75, 75, 74, 74, 74, 71, 67, and 67 degrees of separation, respectively and represented in [Table 1](file:///C%3A%5CUsers%5CHP%5COneDrive%5CDesktop%5CTable%201). These targets serve as hubs at the core of the PPI network, linking together the various nodes. PPI of the top 5 targets were discussed in this chapter.



**Fig. 1 PPI of selected targets involved in PH**

*Analysis of KEGG Pathways and Enrichment of GO Functions*

Bioinformatics enrichment analysis, including a GO analysis of biological processes, molecular functions, and cellular components, and a pathway enrichment analysis of the KEGG, was performed on the potential targets for pulmonary hypertension therapy using the Metascape platform. The species was set to "Homo sapiens" and the significance level was P<0.01. The top 10 targets were selected for further analysis, the data was stored and analysed using a bioinformatics platform. Cytoscape v\_3.9.1 software was used for representing the target networking in this chapter. Using the Metascape system, we analysed 65 CICS therapeutic candidates for migraines in terms of GO and KEGG pathway enrichment. The investigation yielded a total of 419 GO enrichment analysis comprising cellular component analysis (06), biological process analysis (407), and molecular function analysis (06). Results from the top ten GO characteristics were chosen, and the bioinformatics platform was used to preserve and visualize the results. The KEGG analysis yielded a total of 93 pathways was represented in [Fig.2](file:///C%3A%5CUsers%5CHP%5COneDrive%5CDesktop%5CFig.2). The KEGG pathways are mainly related to AGE-RAGE signaling pathway in diabetic complications, Fluid shear stress and atherosclerosis, HIF-1 signaling pathway, positive regulation of oxidoreductase activity, multicellular organismal-level homeostasis, Malignant pleural mesothelioma, vascular process in circulatory system, positive regulation of MAP kinase activity, regulation of body fluid levels, positive regulation of cell growth, maintenance of location, positive regulation of angiogenesis etc.



**Fig. 2 Kegg enrichment pathway**

**EDN1**

Moreover, K198N*(*rs5370*)* polymorphism in the endothelin 1 gene EDN1has been demonstrated to associate with blood pressure reactivity and can result in greater endothelin‑1 (ET-1) synthesis which may favour the development of PAH(10). Therefore, we perform a meta-analysis to evaluate the association of 5-HTT rs25531*,* BMPR2 rs1061157*,*END1 rs5370*,*KCNA5 rs10744676andENG rs3739817 polymorphisms with the risk of PAH. The genotype analysis of the EDN1 [gene-polymorphism](https://www.sciencedirect.com/topics/medicine-and-dentistry/dna-polymorphism) shows statistically significant differences [in patients](https://www.sciencedirect.com/topics/medicine-and-dentistry/inpatient) with PAH compared to healthy individuals. Endothelin-1 (ET-1), a 21-amino acid peptide, is very powerful vasoconstrictor produced in the endothelial cells of vascular smooth muscle. Several studies have demonstrated that ET-1 is involved in PAH pathogenesis due to its vasoconstrictive action and its effects on cell proliferation. Physiologically, ET-1 modulates vascular tone on demand, since it is not stored but produced as necessary. In patients with PAH, the ET-1 concentration in plasma is significantly elevated and its action is essentially mediated by 2 types of receptors: ETA and ETB. The former causes vasoconstriction and the latter, vasodilation as they act as controllers of receptor A, although in pathological situations they may have vasoconstrictive effects too(11).  There is an important relation between ET-1 and endothelial nitric oxide synthase, which, through the production of nitric oxide, acts as a powerful vasodilator. ET-1 is also related to the BMPR2 gene pathway and complex genetic interactions have been described in patients with PAH and mutations of this gene.8 Moreover, inflammation plays a key role in PAH and ET-1 receptor blocking inhibits the activation of some inflammatory pathways.

**Table 1: Degree analysis of top 5 genes**

|  |  |  |  |
| --- | --- | --- | --- |
| **Target gene** | **Degree** | **Closeness Centrality** | **Betweenness Centrality** |
| EDN1 | 77 | 0.811475 | 0.036468 |
| VEGFA | 76 | 0.811475 | 0.033746 |
| ALB | 75 | 0.785714 | 0.023554 |
| AKT1 | 75 | 0.798387 | 0.02952 |
| TNF | 74 | 0.779528 | 0.019528 |

ET-1 is synthesised via multiple proteolytic steps from a precursor protein of 212 amino acids called pre-pro ET-1(12). ET-1 is synthesised via multiple proteolytic steps from a precursor protein of 212 amino acids called pre-pro ET-1. The ET-1 gene, EDN1, located in the 6p24-23 chromosomal region, has 5 exons and is made up of 6386 base pairs. The G>T transversion at +5665 position of the gene, causing the change from Lys (K, lysine) to Asn (N, asparagine) at codon 198 (K198N, rs3570), has been associated with the development of PAH(13). This polymorphism has been associated with blood pressure reactivity, body mass index and ET-1 concentration in plasma. Previous studies have shown that carriers of the T allele have a significant increase in EDN1 action, which could lead to greater ET-1 synthesis and, thus, favour the development of PAH.

NO and EDN1 are two most prominent endothelial mediators. NO is a potent endogenous vasodilator synthesized from L-arginine, catalyzed by the enzyme endothelial NO synthase, encoded by NOS3. EDN1 is produced as a 212 amino acid pre-proendothelin and is processed to a relatively inactive 39 amino acids long big EDN1. The big EDN1 is converted by the membrane-bound endothelin converting enzyme-1(ECE-1) to a 21a.a. functional peptide. Understanding the role of EDN-1 and NOS3 in IPAH has therapeutic significance since EDN1 receptor antagonists and NO agonists are currently few of the best possible options available in treatment of IPAH. Two previously known polymorphisms were detected in EDN1; (a) an “A” insertion(I)/deletion (D) in exon1 at position +138 (rs10478694), the polymorphism is designated as 3A/3A (wild type/deletion), 3A/4A, 4A/4A (mutation/ insertion); and (b) a G/T transversion at position +5665 (rs5370) affecting the 61st nucleotide of exon 5, which substitutes Lys at 198 codon with Asn (K198N). The polymorphism is designated as – Lys198Lys, Lys198Asn, Asn198Asn. [Table 1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3076769/table/tbl1/) gives the allelic and genotypic frequencies of the two EDN1 polymorphisms among controls and patients. No significant deviation from the Hardy Weinberg equilibrium was observed either in patients or control subjects. In rest of the exons of EDN-1, no genetics variations were observed.

**VEGFA**

Pulmonary hypertension (PH) represents a group of highly morbid and lethal disorders characterized by pulmonary vascular dysfunction and right heart failure. It could be caused by *BMPR2* gene variants, pulmonary fibrosis, left heart diseases, and many other pathogeneses(2)**.** and is frequently associated with increased VEGF-A (vascular endothelial growth factor A)/VEGFR2 (vascular endothelial growth factor 2) expression(14). VEGF-A is a major angiogenic growth factor both in health and diseases. After binding to its primary receptor VEGFR2 on vascular endothelial cells, it induces tyrosine phosphorylation of VEGFR2 at specific sites and triggers a complex network of downstream signaling events that are critical for endothelial cell survival, proliferation, migration, and permeability(15, 16).Major phosphorylation sites of VEGFR2 are tyrosines Y1054 and Y1059 that are required for kinase activity and tyrosines Y949 (Y951 in the human), Y1173 (Y1175 in the human), and Y1212 (Y1214 in the human) that on phosphorylation directly bind to differential intracellular adaptor proteins to transduce VEGF-A signals. Phosphorylation of Y1175 and subsequent activation of the PLCγ-ERK axis are required for endothelial cell development and proliferation (17, 18) whereas phosphorylation of Y1214 has been described to critically regulate CDC42-mediated front-back polarization during endothelial cell migration by recruiting NCK and also endothelial cell proliferation by activating GRB2/ERK and PI3K/AKT pathways(19, 20). In the present study, we found that PH in both human patients and hypoxia-induced mouse models was associated with VEGF-A–induced vascular leak.

The vascular endothelial growth factor (VEGF) is the main angiogenic factor and is indispensable for the process of normal angiogenesis. The overexpression of VEGF is associated with proliferation of ECs in severe PAH(Sakao and Tatsumi,2011). VEGF and its receptor showed increased expressions in animal models (hypoxia or MCT-induced PH), and elevated levels of plasma VEGF were also discovered in PAH patients (voelkel and Gomez-Arroyo,2014). VEGFprotein levels in the lungs of TGF-α mice*.* Lung VEGF-A protein levels were decreased by 28% in adult and 45% in 2-day-old TGF-α mice compared with age-matched WT controls. Western blot analysis also showed that VEGF164 protein levels were reduced in the lungs of 2-day and adult TGF-α mice. The VEGF family member placental growth factor (PlGF) binds exclusively to Flt-1, which leads to pro-angiogenic signalling through several mechanisms, including direct intracellular activation of Flt-1 and downstream target genes, and transphosphorylation of VEGFR-2 by activated Flt-1, thereby increasing the response to VEGF. Moreover, the PlGF/VEGF-A heterodimer can bind and activate Flt-1 and induce Flt-1/VEGFR-2 dimerisation.

**ALB**

Decreased ALB was associated with decreased PAH survival(21,22).Increased bilirubin level was correlated with an elevated mortality risk of PAH(23,24). Only ALB was correlated with the length of ICU stay in the total PPH group. ALB independently served as a risk variable for hospital mortality and 90-day mortality and was significantly associated with 90-day and 4-year survival rates in both PPH and PPH without liver disease (3) Patients who underwent routine blood tests 24 h upon admission, and the tested indicators included white blood cell(WBC), serum sodium, serum potassium, platelets, blood urea nitrogen (BUN), creatinine, total bilirubin, blood alanine transaminase(ALT), blood aspartate transaminase(AST), and blood albumin(ALB),The criteria for exclusion used in this research were patients who did not develop PPH; patients under the age of 18; patients whose blood test results were not complete; and patients with PPH suffering from systemic immune system diseases, active infectious diseases; other important functional organs severely damaged, malignant tumors, etc. The brief mechanism of pulmonary hypertension with screened genes was illustrated in the [Fig. 3](file:///C%3A%5CUsers%5CHP%5COneDrive%5CDesktop%5CFig.%203).

**AKT1**

The Akt signaling pathway plays an important role in regulating cell proliferation, migration, and apoptosis, much of which has been linked to subsequent activation of the mTOR, an important downstream signaling protein(25, 26). Distinct actions of Akt isoforms in vascular tissue have also been noted, such that microvascular permeability and edema formation are attenuated in *Akt1−/−* mice and by silencing *Akt1* in pulmonary vascular endothelial cells, whereas Akt2 does not appear to play a significant role(27). There are three members in the Akt kinase family, Akt1, Akt2, and Akt3, which share a high degree of homology. Akt1 and Akt2 are broadly expressed in many tissue and cell types, whereas Akt3 is predominantly expressed in brain tissue(28, 29). Each of the Akt isoforms has a distinct but overlapping function in the regulation of cell proliferation, cell apoptosis, protein synthesis, and the cell cycle(30). Blockade of the Akt/mTOR signaling with rapamycin or an Akt inhibitor can significantly attenuate PASMC proliferation(31). Deletion of PTEN in mouse smooth muscle cells results in vascular remodeling(32), and exposure of PTEN conditional knockout (KO) mice to hypoxia causes severe pulmonary hypertension(33).



**Fig.3 The mechanism of pulmonary hypertension with screened genes**

In this study, we hypothesized that PTEN/Akt/mTOR signaling contributes to the development and progression of pulmonary hypertension, and deletion of different Akt kinase isoforms (e.g., Akt1 or Akt2) may have distinctive effects on the development and progression of pulmonary vascular remodeling in animals with experimental pulmonary hypertension. Asymmetric dimethylarginine (ADMA) induces the mitochondrial translocation of endothelial nitric oxide synthase (eNOS) through the nitration-mediated activation of Akt1. However, it is recognized that the activation of Akt1 requires phosphorylation events at threonine (T) 308 and serine (S) 473. Thus, the current study was performed to elucidate the potential effect of ADMA on Akt1 phosphorylation and the mechanisms that are involved. Finally, we found that the mitochondrial translocation of eNOS in our lamb model of pulmonary hypertension is associated with increased Akt1 and eNOS phosphorylation and reduced Akt1-CTMP protein interactions. In conclusion, our data suggest that CTMP is directly involved in ADMA-induced Akt1 phosphorylation in vitro and in vivo, and that increasing CTMP levels may be an avenue to treat pulmonary hypertension.

**TNF**

We confirmed that caspase-11 and its human homolog caspase-4 were activated in PAH animal models and TNF (tumor necrosis factor)-α–induced human pulmonary arterial endothelial cells. Moreover, knockdown of caspase-4 repressed the onset of TNF-α–induced pyroptosis in human pulmonary arterial endothelial cells and inhibited the activation of pyroptosis effector GSDMD (gasdermin D) and GSDME (gasdermin E). Endothelium dysfunction can occur in response to inflammatory mediators, such as TNF-α, which is elevated in the plasma of patients with PAH and animal models, and considered to be a classical pro-PAH factor, we use TNF-α as a stimulator of HPAECs to conduct mechanism research of caspase-4/11–mediated pyroptosis(34, 35). Notably, arising evidence also indicated that the function of TNF-α was associated with pyroptosis through caspase-3 cleavage of GSDME (gasdermin E) (36); therefore, this study aims to investigate the role of caspase-11–mediated pyroptosis in the PAH pathogenesis and its specific mechanism on endothelial function stimulated by TNF-α. Here, we demonstrated that caspase-4/11 was activated in the PAH animal models and TNF-α–induced HPAECs, knockout, or pharmacological inhibition of caspase-11 could protect against PAH.

Moreover, HIF-1α can also be stabilized by inflammatory molecules, such as IL-1β, NF-κB and TNF-α. NF-κB has been considered the master regulator of inflammation under hypoxic conditions since NF-κB can stabilize HIF-1α when it is released from inhibitory kinase b (IKb) through nuclear factor kinase subunit b (Ikkβ) activation. An interesting study in rats exposed to chronic hypobaric hypoxia showed an increase in 12(s)-hydroxyeicosatetraenoic acid (12(s)-HETE) expression in the lung, which was produced by leukocyte-type 12 lipoxygenase (12-LO) activation. This activation contributes to inflammatory pathways and the activation of ERK1/2 and p38 MAPK in smooth muscle cells (SMCs). Then, the proliferation process is stimulated, and HAPH subsequently develops. Therefore, both acute and chronic exposure to high altitudes activate inflammatory pathways that contribute to the development of pulmonary high-altitude illnesses such as HAPE and HAPH, which will be discussed in the following sections. Patients with HAPE have increased TNF-α and IL-6 levels in serum. Then, Sharma et al. determined that the levels of TNF-α were increased in the blood of individuals with HAPE, and this alteration could have a role in lung permeability in patients with HAPE(37).

**Conclusion**

This chapter uncovered a set of genes that the network pharmacological approach to hypertension predicted would have a role in the disease. Using this method, we observed that the majority of AKT1's protein-protein interactions compare with other genes. We propose that this study provides a novel approach to investigating issues of this nature and adds to our understanding of the genes involved in hypertension by shedding light on their functional and pathway connections. More preclinical and clinical research is needed to corroborate our findings, but our study suggests that the AKT1 gene, via multiple pathways, may be a unique treatment method for pulmonary hypertension.

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