

A Study on the Molecular Mechanism of High Altitude Heart Disease in Children

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Objective: High altitude heart disease (HAHD) is a common pediatric disease in high altitude areas. It usually occurs in people who have lived for a long time or have lived for more than 2500m above sea level. Its common inducement is respiratory tract infection. The clinical differential diagnosis is difficult because the symptoms of HAHD are similar to those of congenital heart disease; Due to the limitation of medical conditions, many patients are in the state of losing follow-up or not seeking medical treatment, resulting in poor prognosis of HAHD and becoming a high-altitude disease with high mortality. Clarifying the molecular mechanism of HAHD, developing early molecular screening technology and accurate treatment methods of HAHD are the key to improve the ability of prevention and treatment of HAHD.

Methods: First, the literature in the PubMed and CNKI databases were screened based on keywords and abstracts. Then, the literature for the study was identified based on the fitness between the content of the literature, the research objectives, and the timeliness of the literature. Finally, a systematic molecular mechanism of HAHD was established by investigating the literature and sorting out the genetic adaptations of Tibetan populations compared with low-altitude populations that migrated to the plateau.

Results: With the investigation of the 48 papers screened, it was found that genes capable of enhancing the hypoxic ventilatory response and resistance to pulmonary hypertension were all correlated with the hypoxia-inducible factor (HIF) pathway, consisting mainly of three pathways, HIF-1 α , HIF-2 α , and NO.

Conclusion: The low prevalence of HAHD in Tibetan aboriginal children was mainly due to the genetic adaptation of the Tibetan population to the high altitude environment, which coordinated the cellular response to hypoxia by regulating the downstream hypoxia control genes in the HIF pathway.

Keywords: altitude sickness, pediatric, high altitude heart disease, HAHD, molecular mechanism

Introduction

In 1995, the Chinese Medical Association adopted the "Nomenclature, Typing, and Diagnostic Criteria for High Altitude Heart Disease (HAHD) in China" at the Third Symposium on High Altitude Medicine and proposed the diagnostic features of HAHD¹ as follows: 1) the disease is most common in areas with an altitude of ≥ 3000 m, and a few susceptible individuals may develop the disease at an area with an altitude of approximately 2500 m above sea level; 2) the highest incidence in children born at low altitudes and raised at high altitudes and lowland children born in highland areas; 3) clinical symptoms and signs of pulmonary artery hypertension (PAH) and congestive heart failure; 4) prominent pulmonary artery, dilated right ventricle, and spherical heart shadow on chest X-rays; 5) right axis deviation, right ventricular hypertrophy, and right bundle branch block in Electrocardiograph (ECG); 6) significantly elevated pulmonary artery pressure in cardiac ultrasonography; 7) alleviation of symptoms and signs with the patient moving to an area of normal sea level or lowland; 8) without congenital heart disease and acquired organic lesions of the heart. In 2013, the Fifth World Symposium on PAH classified PAH with similar pathological manifestations, hemodynamic characteristics,

and treatments into five types, of which HAHD is classified in the third major category (3.6 Long-term exposure to high altitude),² and is a common cardiovascular disease in high altitude. According to existing epidemiological studies on HAHD, more than 140 million people worldwide live at high altitudes, and more than 40 million people come to high altitudes for various reasons.³ The incidence of high altitude sickness due to reduced oxygen partial pressure among people entering high altitude from the plains increases every year.⁴ In 2020, Tibetan children aged 0–14 years accounted for 26.48% of the total population in Tibet. The prevalence of HAHD in children is approximately 1.16% in males and 0.75% in females, and the mortality is 0.8%. Children under two years old are most susceptible, but children of other ages can also develop HAHD, and the onset is mostly sub-acute. The lower prevalence of HAHD in Tibetans relative to the Han population who migrated to high altitude may be correlated with the family genetics of adaptation to high altitude.

In recent years, the diagnosis rate of HAHD in children has been increasing with the continuous improvement of medical conditions and the widespread clinical application of auxiliary examinations, such as color Doppler echocardiography.^{5,6} However, the pathogenesis of HAHD remains unclear, and it occurs mostly in infants and children who are prone to respiratory infections, which can further trigger and aggravate the onset of HAHD in children. Malnutrition and anemia are also factors that exacerbate HAHD in children. In addition, HAHD has a similar phenotype to pulmonary heart disease, exudative pericarditis, cardiomyopathy, congenital heart disease, and rheumatic heart disease, leading to a tendency to underdiagnose and misdiagnose. In Shigatse people's Hospital, since 2017, about 300 children have been hospitalized due to respiratory diseases and finally determined as HAHD, and the in-hospital mortality rate is 1.6 ~ 2.2% (based on the data statistics of the in-hospital Information Department). However, we believe that the incidence rate and mortality rate of this disease are underestimated due to the limited medical and living conditions. Many HAHD patients do not come to the hospital for treatment or misdiagnosis, especially in remote areas/high altitude areas and pastoral areas, which are limited by natural conditions/economic conditions/disease recognition, and there are still a certain number of children who are not treated at the present time, so the mortality rate in the real world is still unknown. At present, the clinical diagnosis of high altitude heart disease in children is mostly based on clinical symptoms and epidemiology. The poor prognosis of the disease is also related to the failure of local cardiac ultrasound and other related medical examinations. Without systematic and comprehensive examination, there will be no better treatment scheme and health management.

In recent years, with the development of various molecular detection technologies, accurate detection of complex diseases has become possible. Systematic analysis of the molecular mechanism of diseases will help clinical researchers find that high sensitivity, high specificity and convenient molecular detection technologies can be popularized in Tibetan areas. However, different studies on HAHD have their own emphases. At present, there is no complete molecular mechanism signal pathway of HAHD. Moreover, the study of plateau disease is not as extensive as that of common diseases in the plain, and some mechanisms are not supported by perfect human clinical data. Therefore, this study is mainly based on the comprehensive analysis of literature and combined with the data of high altitude animal to deduce the potential complete molecular mechanism signal network of HAHD, provide important clues for improving the treatment effect of HAHD and exploring efficient treatment methods, develop new technologies for screening, accurate diagnosis and treatment of HAHD, reduce the infant mortality of HAHD in Tibet and improve the health level of the population.

Study Subjects and Methods

In the present study, the signaling pathways of HAHD-related molecular mechanisms were established through systematic literature analysis, aiming to provide directions for early diagnosis and precise treatment for HAHD. The literature screening was carried out in three stages. In the first stage, 601 documents were retrieved: 165 records and 18 records were obtained by searching the keywords “HAHD” and “(HIF) and (high altitude)”, respectively, in the database of CNKI. The PubMed database was then searched with “(high altitude heart disease) and (children)”, as well as “(HIF) and (high altitude)”, respectively. The search yielded 124 and 294 records, respectively. One hundred twenty documents were retrieved in the second stage. The abstracts of each article were browsed and further screened for literature containing relevant gene signaling pathways and therapeutic content. Forty-eight documents were retrieved in the third stage: the full text of the

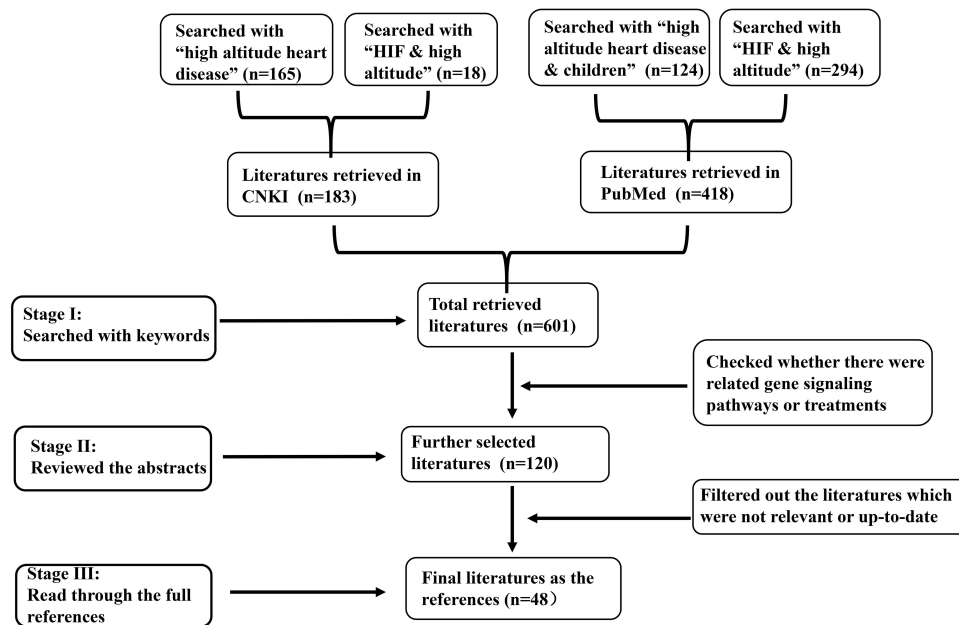


Figure 1 Flow chart of literature screening. n represented the number of screened paper.

literature was read in detail, and finally, the references for the present study, based on the suitability of the content of the literature to the research objectives and the timeliness of the literature, were screened out, as illustrated in [Figure 1](#).

Results

Related Genes in Signaling Pathways

A detailed reading of the final 48 screened articles revealed that genes that enabled the Tibetans to adapt to chronic hypoxia at high altitude and enhance hypoxic ventilatory response and resistance to PAH were all correlated with the hypoxia-inducible factor (HIF) pathway when compared with the low altitude populations that migrated to the high

Table 1 The Information on Relevant Genes Involved in the Signaling Pathway of the Molecular Mechanism in High Altitude Heart Disease

Gene	Full Name	Location	Full Length (kb)	Number of Exons
NOS3 (eNOS)	Nitric oxide synthase 3	7q36.1	23.6	28
NOS2 (iNOS)	Nitric oxide synthase 2	17q11.2	43.8	27
HIF-1 α	Hypoxia-inducible factor-1 α	14q23.2	52.7	16
HIF-1 β	Hypoxia-inducible factor-1 β	1q21.3	66.9	25
ET-1	Endothelin-1	6p24.1	40.7	9
HIF1AN	Hypoxia inducible factor-1 subunit alpha inhibitor	10q24.31	24.1	8
PTEN	Phosphatase and tensin homolog	10q23.31	108.3	10
PI3K	Phosphatidylinositol 3'-kinase			
PHD2 (EGLN1)	Prolyl hydroxylase domain	1q42.2	58.5	5
EP300	E1A binding protein p300	22q13.2	87.5	31
VEGF	Vascular endothelial growth factor	6p21.1	16.3	9
VEGFR	Vascular endothelial growth factor receptor	4q12	47.1	30
PPARA	Peroxisome proliferators activated receptor α	22q13.3	93.2	14
ANGPTL4	Angiopoietin-like 4	19p13.2	10.2	7
HIF-2 α (EPAS1)	Endothelial PAS domain protein 1	2p21	89.3	17
EDNRA	Endothelin receptor type A	4q31.22-q31.23	63.9	9

altitude.⁷ The genes involved in these pathways were as follows: NOS3 (eNOS), NOS2 (iNOS), HIF-1 α , HIF-1 β , ET-1, HIF1AN, PTEN, PI3K, PHD2 (EGLN1), EP300, VEGF, VEGFR, PPARA, ANGPTL4, HIF-2 α (EPAS1), and EDNRA. The details are demonstrated in Table 1.

The Molecular Mechanism of the HIF Pathway in HAHD in Children

Tibet is located in the southwest of the Qinghai Tibet Plateau, with an average altitude of more than 4000m. The pathological mechanism of HAHD is that hypoxia, cold, dryness and other factors caused by high altitude environment are easy to cause alveolar hypoxia, followed by hypoxic pulmonary vasoconstriction and hypoxic pulmonary hypertension. Hypoxic pulmonary hypertension and thickening or reconstruction of pulmonary arteriole wall are the main pathogenesis of pulmonary hypertension. Chronic hypoxia will lead to dysfunction of pulmonary vascular endothelial cells, increase the release of vasoconstrictor and pro proliferator factors, and lead to pulmonary vascular remodeling. Long-term hypoxemia, excessive red blood cell proliferation and hypoxic pulmonary hypertension gradually aggravate the pressure load of the right heart and produce compensatory hypertrophy of the right ventricle. When the course of disease continues to develop, the cardiac reserve further decreases, which can damage cardiomyocytes, weaken myocardial contractility and reduce cardiac output, and finally lead to right heart failure and premature death.⁸

The HIF signaling pathway is involved in vascular development, regulating oxygen transport, and efficient nutrient utilization, as well as controlling the response to hypoxia. In addition, our previous studies in the animal models, including the Tibetan mastiff and Tibetan pig, have found that some important genes in the HIF/VEGF signaling pathway, such as EPAS1, were mutated, and there existed significant differences in the expression of these signaling pathway-related genes when compared with animals in the plains,^{9,10} suggesting that the cardiovascular system of mammals in high altitudes may undergo specific selective evolution of the cardiovascular system through adaptation in response to the high altitude climate. The evolution may provide a protective mechanism for the cardiovascular system under hypoxia and is closely correlated with high altitude adaptation and the occurrence of some high altitude diseases in Tibetan populations. Hypoxia activates a variety of general and specific signaling pathways in the cell, and these

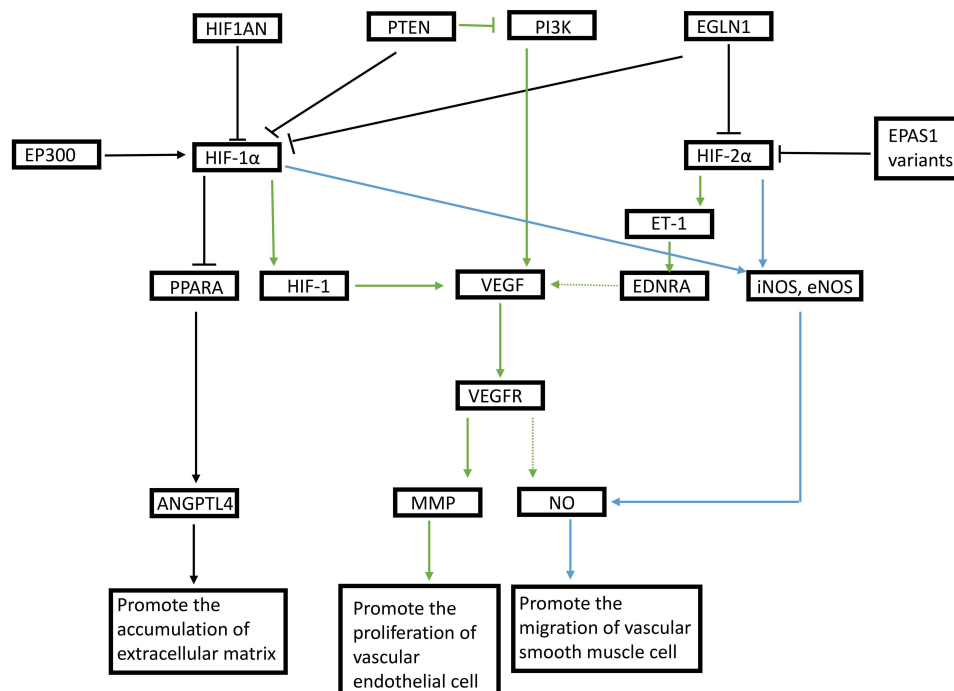


Figure 2 The molecular mechanism of the HIF pathway in high altitude heart disease in children.

Notes: The T-shaped arrow “⊥” represented inhibition, and the single arrow “→” represented promotion. The solid line represents the pathway with sufficient evidence, and the dotted line indicates the pathway with weak evidence.

interrelated and interdependent signaling pathways might determine the overall cellular response at each time interval¹¹ (Figure 2).

Under hypoxia, HIF1AN negatively regulates HIF-1 α , PTEN negatively regulates HIF-1 α and VEGF, and EGLN1 negatively regulates the α subunit of HIFs in an oxygen-dependent manner. The EPAS1 variant negatively regulates HIF-2 α , and EP300 activates HIF-1 α via Asn in trans, resulting in a large increase in hypoxia-related substances. On the one hand, the expression levels of VEGF were positively correlated with the expression of HIF-1 α or HIF-2 α ,¹² and hypoxia is the primary stimulus for VEGF production. While PI3K promotes VEGF expression,¹³ the heterodimeric HIF-1 formed by dimerization of HIF-1 α and HIF-1 β and the hypoxia-responsive element (HRE) is the cis-transcriptional element for VEGF under hypoxia. The tandem VEGF-HREs are ideal candidates for developing hypoxia/HIF-regulated gene delivery vectors. In addition, HIF-2 α promotes VEGF expression through the upregulation of EDNRA by ET-1. Then, the VEGF protein binds massively to VEGFR on the endothelial cell membrane via Flt-1, increasing the contents of the downstream factor NO to cause vasodilation, promoting vascular endothelial cell proliferation, and stimulating MMP activity promotes migration of vascular smooth muscle cells. On the other hand, HIF-1 α inhibits the transcription of PPARA, which further inhibits the transcription of ANGPTL4, thereby decreasing the hemoglobin concentration, allowing cells to adapt to the chronic hypoxic environment and promoting the accumulation of extravascular matrix. Finally, both iNOS and eNOS are target genes of HIF α ¹⁴ and are directly regulated by HIF-1 α and HIF-2 α to produce NO, the essential vasodilatory molecule.¹⁵

Ultimately, under the joint action of the above genes, by promoting extravascular matrix accumulation, vascular endothelial cell proliferation, and vascular smooth muscle cell migration, as well as increasing vasodilation and blood flow, it will cause VSMC to accumulate in large quantities in the intima with the formation of connective tissue. These will promote the formation of muscle tissue vessels and neointima, reconstruct the ischemic pulmonary vasculature, adapt to the hypoxic environment at high altitude, and achieve effective self-adaptation to HAHD.¹⁶

The Molecular Mechanism of the NO Pathway

NO is a potent endogenous vasodilator that contributes to the distribution of blood from poorly ventilated areas to well-ventilated areas, thus maintaining a normal ventilation-perfusion equilibrium.¹⁷ High NO levels promote vasodilation and increase blood flow, and elevated circulating NO levels increase cellular utilization of oxygen, which is an adaptive physiological feature in Tibetan populations. A previous study has shown¹⁸ that the NO levels in 88 Tibetans living at an altitude of 4300 m were 10 times higher than those in 50 Europeans and Americans living at an altitude of 203 m. NO is not only involved in the process of blood flow control but also a regulator of blood oxygen utilization. eNOS and iNOS both contain HRE motifs in the gene promoter region.¹⁴ Hypoxia causes the upregulation of iNOS, while HIF-1 α and HIF-2 α promote the synthesis of NO products in vivo, thereby increasing NO products in tissues and cells (the blue pathway in Figure 2). NO contributes to a blunted response to hypoxia by inhibiting mitochondrial oxygen consumption, thereby providing more oxygen to proline hydroxylase to reduce the levels of HIF protein induced by hypoxia.

The Molecular Mechanism of the HIF-1 α Pathway

HIF-1 consists of two subunits, HIF-1 α and HIF-1 β ,¹⁹ and is present in the organism and tissue cells.²⁰ Among them, HIF-1 α is the most important regulator to maintain oxygen homeostasis, which can promote the binding of HIF-1 to HRE under hypoxia, increase the stability of HIF-1, relax smooth muscle, and prevent PAH. During chronic hypoxia in the alveolar tissue, HIF-1 expression is significantly increased in the pulmonary tissue, leading to elevated intracellular Ca²⁺ and ET-1 in pulmonary artery smooth muscle cells during the prolonged hypoxia.¹⁴ While ET-1 is one of the most effective vasoconstrictors,¹⁷ it may cause vasoconstriction, promote the proliferation of pulmonary vascular smooth muscle cells, and activate HIF-1 in pulmonary artery smooth muscle cells leading to the development of PAH.²¹ HIF1AN belongs to the 2-ketoglutarate-dependent dioxygenase superfamily, which regulates the functional activity of protein interactions and target substrates through asparagine-based hydroxylation,²² and can block the interaction between HIF and the co-activator CBP/p300.²³ PTEN, a phosphoinositide phosphatase involved in growth and metabolic signaling transduction,²⁴ can decrease the stability of hypoxia-mediated HIF-1 α , negatively regulate PI3K-mediated apoptosis, and inhibit VEGF expression via PI3K.²⁵ EGLN1 is a molecular oxygen sensor that regulates the HIF transcriptional pathway,²⁶ and the hydroxylase activity is inhibited by hypoxia leading to the accumulation of HIF, which can activate

hundreds of downstream target genes and induce erythropoiesis.¹⁵ Simonson et al²⁷ found that the EGLN1 and PPARA haplotypes were significantly correlated with decreased hemoglobin concentrations specific to the high altitude population. There existed a relatively significant allelic difference in EP300 between Tibetan and lowland populations.²⁸ Zheng et al¹⁴ found that Tibetans have a positive signal for Darwinian selection on EP300, and the selection function on EP300 might be correlated with the role in the hypoxic pathway. VEGF exerts vasodilatory effects through the downstream factor NO, which promotes vascular endothelial cell proliferation, vascular smooth muscle cell migration, and extravascular matrix accumulation, and has a vital role in pulmonary vascular remodeling.²⁷ It was found that there existed a HIF-1 binding site (HRE) within the 5' end enhancer of VEGF. Although PPARA was not considered a candidate gene for high altitude acclimation, it interacts with components of the HIF pathway. The PPARA gene has a DNA common motif for HIF-1.²⁹ Recently, Narravula et al²⁹ found that hypoxia-induced PPARA downregulation was correlated with HIF-1 α induction and could protect the epithelial cells from PPARA-induced ICAM-1 agonist amplification, for the first time identifying the downregulation pathway mediated by HIF-1 binding. ANGPTL4 is correlated with factors regulating angiogenesis, involved in angiogenesis and vascular permeability,³⁰ and is also a target of PPARA, the transcription of which is regulated by multiple transcription factors, including PPAR α , PPAR β/δ , PPAR γ and HIF-1 α .²¹

In summary, under hypoxia, HIF-1 α might be regulated by various related factors (the black pathway in Figure 2): the oxygen-sensitive enzyme HIF1AN causes hydroxylation of HIF-1 α , and other anchor proteins repeat structural domain proteins at the asparagine residues.²² PTEN may completely inhibit the stabilization of HIF-1 α through hypoxia.²⁴ PHD2 may negatively regulate HIF-1 α in an oxygen concentration-dependent manner. EP300¹⁴ is a hypoxia switch that acts through specific recognition and hydroxylation of asparagine (L-Asparagine, Asn) to activate HIF-1 α in trans. Overall, under hypoxia, the inhibition of HIF-1 α degradation would lead to the accumulation and formation of heterodimers with HIF-1 β . Subsequently, this HIF complex would bind to the HRE at the 5' end of VEGF to promote the transcription and expression of VEGF and promote structural remodeling of the pulmonary vasculature and right ventricle.³¹ In addition, there exists a previously unappreciated HIF-1 α binding site at positions 832–836 (relative to the first methionine codon) of the PPARA antisense strand (the DNA common motif 59-ACGTG-39).²⁹ EMSA analysis revealed that environmental hypoxia induced the binding of HIF-1 α to the HIF-1 shared structural domain of PPARA, along with intranuclear aggregation of HIF-1, and antisense depletion of HIF-1 α resulted in a loss of PPARA down-regulation. ANGPTL4 is regulated by PPARA, inhibition of PPARA transcription would lead to inhibition of ANGPTL4 transcription as well. Under the combined action of the above genes, it can relax the smooth muscle by promoting vascular endothelial cell proliferation, vascular smooth muscle cell migration and extravascular matrix accumulation, and then to achieve the goal of promoting pulmonary artery relaxation, so as to prevent the occurrence of pulmonary hypertension.

The Molecular Mechanism of the HIF-2 α Pathway

EPAS1 achieves the *in vivo* cell adaptation to high altitude hypoxia by encoding HIF-2 α , which has the strongest selection signal in previous genome-wide scans in Tibetans¹⁵ and is a functional contributor to hypoxia tolerance in Tibetans.³² Functional changes in EPAS1 in hypoxic adaptation strategies occur at the transcriptional level, with EPAS1-adapted variants resulting in reduced EPAS1 expression under hypoxia compared with the wild-type controls. According to data from Tibetan endothelial cells and placenta, the reduction in the expression level of EPAS1 was 31.3% and 27.1%, respectively, which might be the molecular basis of the retarded hypoxic response in Tibetans.¹⁵ All identified EPAS1 variants are located in non-coding regions (mainly in introns), suggesting that they might affect the regulation of EPAS1 at the transcriptional level.²⁸ The EPAS1 mutation provides a protective mechanism for the cardiovascular system under hypoxia by reducing blood flow resistance to avoid high altitude responses. A candidate gene study comparing the Andean Native American and Lowland Native American populations identified positive selection signals for many genes, including EDNRA, PRKAA1 (protein kinase, adenylate activation, and α 1 catalytic subunit), and NOS2A,³² in which the DNRA variants evolve by adaptive selection were also identified in Tibetan populations and Tibetan sheep. According to Lee et al³³ who suggested that “EDN1 might significantly increase CDH2 and VEGF expression through EDNRA” in a mechanistic analysis, it could be confirmed that EDNRA might positively regulate VEGF. EDNRA encodes the receptor for endothelin-1 (ET-1), and controls the production of type A endothelin receptors, which are part of the

endothelin system.³⁴ EDNRA is expressed primarily in vascular smooth muscle cells and has no known effect other than vasoconstriction.³⁵ ET-1 is important as a potent vasoconstrictor involved in vascular homeostasis in HIF-2 α pathway.

In summary, the molecular mechanisms of the HIF-2 α pathway were as follows (the green pathway in Figure 2): EGLN1 might be the negative regulator of EPAS1 under hypoxia.³⁶ Subsequently, the binding of VEGF proteins to VEGFR on the endothelial cell membranes in large amounts under the combined effect of regulation of Flt-1 expression on the endothelial cell membrane through autocrine or paracrine responses, as well as the autophosphorylation of VEGFR under the action of PI3K, would increase the content of the downstream factor NO to cause vasodilation and promote vascular endothelial cell proliferation.^{9,37} VEGF stimulates endothelial cells to produce MMPs, and VEGF and MMP-9 play a synergistic role in angiogenesis. MMPs are a family of metalloendopeptidases that cleave protein components of the extra-cellular matrix (ECM) and basement membrane of the endothelial cell as well as play a pivotal role in angiogenesis.³⁸

The Treatment of HAHD in Children

The primary pathogenic mechanism of HAHD in children⁸ is the thickening or reconstruction of the pulmonary arteriole walls due to low pressure and hypoxia. Therefore, the key in the treatment of HAHD in children is to reduce the pulmonary artery pressure and cardiac afterload.³⁹ Currently, there is no specific drug for HAHD, and in clinical practice, the primary treatment is prevention and symptomatic treatment, including continuous oxygenation,⁴⁰ prevention and treatment of respiratory tract infections, reduction of pulmonary artery pressure, reduction of right heart load by diuretics, and if necessary, sedation, infection control, diuresis to reduce cardiac load, and correction of water-electrolyte acid-base balance disorders in children.⁴¹ The most common predisposing factor for HAHD in children is respiratory tract infection, and its prevention and treatment in children can effectively reduce the incidence of HAHD.⁴²

Li et al⁴³ found that betaloc combined with captopril was effective in treating HAHD in children, which could effectively control the progression of myocardial remodeling, repair and improve the myocardial contractile function in children, and result in a better prognosis. Phentolamine and dobutamine were administered in addition to the conventional treatment by Wang et al⁴⁴ in clinical practice, which could increase cardiac output as well as pumping volume with significant clinical efficacy. The leading prominent clinical symptom of HAHD is PAH. The phosphodiesterase-5 inhibitor (PDE-5i), such as sildenafil and tadalafil, endothelin receptor antagonists (ERA) such as bosentan, ambrisentan, and macitentan, and prostaglandin I₂ (PGI₂) analogs such as epoprostenol, travoprost, iloprost, and selective prostacyclin receptors (IPR) selexipag have certain clinical applications in HAHD.⁴⁵ Suolangdeji⁴⁶ found that sildenafil, as a first-line drug in the treatment of PAH, significantly improved the symptoms of heart failure and activity tolerance in patients with HAHD combined with severe PAH, improved the right ventricular function, and increased coronary perfusion by enhancing the NO and cyclic guanosine acid signaling system on the pulmonary artery smooth muscle. The main molecular mechanism is shown in Figure 3. In the NO-sGC-cGMP pathway, the soluble guanylate cyclase (sGC) catalyzes the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), the main mediator in the NO signaling transduction, thereby increasing NO levels. In addition, the expression of PDE-5 was upregulated in pulmonary vascular smooth muscle cells (VSMC) in patients with PAH. Under hypoxia, K⁺ channels are inhibited, while Ca²⁺ inward flow leads to the proliferation of pulmonary artery smooth muscle cells and promotes the production of vasoconstrictor substances. Meanwhile, PDE-5 is an excellent target. PDE-5i sildenafil increases the cGMP content in the pulmonary vascular smooth muscle by inhibiting the activity of PDE-5 to activate protein kinase G (PKG), which increases the K⁺ channel opening and hyperpolarizes the cell membrane. Ultimately, Ca²⁺ inward flow is inhibited. Decreased intracellular Ca²⁺ concentration promotes pulmonary smooth muscle relaxation and vasodilation, decreases pulmonary vascular resistance, lowers pulmonary artery pressure, and increases cardiac output. It also could promote vasodilation and inhibit smooth muscle cell proliferation in the pulmonary circulation by inhibiting the degradation of cGMP and increasing the effect of endogenous NO. Xia et al⁸ further demonstrated that sildenafil effectively reduced pulmonary artery pressure and improved cardiac function in children with HAHD combined with severe PAH. Moreover, Jiang et al⁴⁷ described that vardenafil, a novel PDE-5i, could reduce arterial pressure (PA) and venous pressure (PV) by inhibiting PDE-5 activity, which was effective in treating PAH in children, providing a good prospect for the pharmacological treatment of HAHD in children. In addition, Li et al⁴¹ found that the application of stilbestrol in addition to the

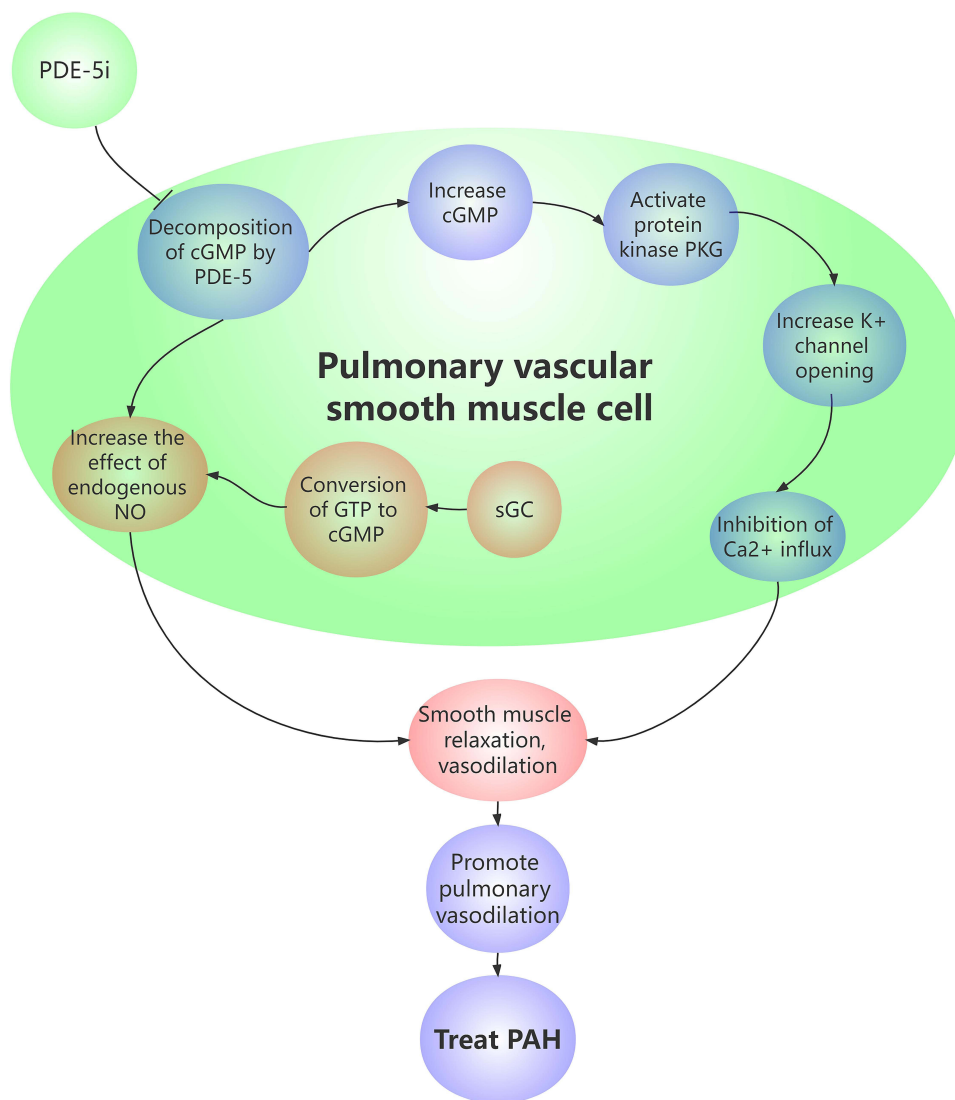


Figure 3 The molecular mechanism of PDE-5i in the treatment of PAH.

Notes: The T-shaped arrow “⊥” represented inhibition, and the single arrow “→” represented promotion.

conventional treatment in HAHD in children could effectively lower the pulmonary artery systolic pressure⁴⁸ and significantly improve the cardiopulmonary function in children with high safety and no adverse effects. However, since patients with HAHD are often in a hyper-coagulation status and are at risk of thrombosis, clopidogrel and aspirin are often administered to improve the hyper-coagulation status and to effectively reduce the occurrence of thrombosis.⁴⁹

Summary and Prospects

The low prevalence of HAHD in Tibetan aboriginal children reflected the genetic adaptation of the Tibetan population to the high altitude environment. HIFs might play an essential role in oxygen homeostasis by promoting tissue oxygenation under hypoxia. HIFs could coordinate the cellular response to hypoxia by regulating the downstream hypoxia control genes. Within the broad framework of complete inhibition of HIF-1 α and HIF-2 α expression by HIF1AN, PTEN, and PHD2 through hypoxia, genes such as PI3K, iNOS, and eNOS could synergistically regulate PPARA and VEGF to control the levels of ANGPTL4, MMP, and NO, thus promoting arterial smooth muscle and vasodilation, increasing blood flow and providing effective prevention and treatment for HAHD.

In recent years, with the intensive investigation in the field of the HIF pathway, the treatment for HAHD has become increasingly effective. Although the clinical efficacy in children has been improved as well as improvement in the overall

survival quality and rates under the guidance of clear mechanisms, the treatment in children is not optimistic due to the specificity in age, growth, and development, and treatment tools still need to be improved. In the future, more research and technological tools are expected to help the early diagnosis or screening of HAHD and to promote the transformation of the traditional treatment-centered medical model to a prevention-centered medical model.

In addition, although we have consulted a lot of literature, the research on plateau disease is not as extensive as that on common diseases in the plain. Some mechanisms are not supported by sufficient perfect data, and there are still uncertainties in the construction of signal network of HAHD mechanism. For example, we speculate the mechanism of EDNRA to VEGF and VEGFR to go through the conclusions of researchers' heart experiments in Tibetan sheep and Tibetan pigs under hypoxia in recent years. At the same time, in order to improve the mechanism, we focused on the genes with high frequency in the signal pathway of HAHD mechanism (HIF-1 α , HIF-1 β , VEGF, EP300, EPAS1, EGLN1) and related mechanisms were studied. However, some studies have not been included in the analysis. For example, we found that ET-1 can act on HIF-1, but how to regulate HIF-1 α There is not enough theoretical support for the signal pathway, so we have not put ET-1 into HIF-1 α for the time being in the signal path. With the accumulation of future research and the support of more experimental data, we will continue to improve the molecular mechanism and signal pathway of HAHD.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki (as was revised in 2013). The study was approved by Ethics Committee of the People's Hospital of Shigatse (No.2019RSYLL003). Written informed consent was obtained from all participants.

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Disclosure

The authors declare that they have no competing interests.

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