Potential Genetic Contributions to Nonidiopathic, Nonfamilial Pulmonary Hypertension

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The recent Venice Classification of pulmonary hypertension[1] includes diseases that are definitely or possibly genetic and associated with the development of pulmonary hypertension. These include "other" or "miscellaneous" diseases, including Gaucher disease, hemoglobinopathies, myeloproliferative disorders, thyroid disorders, sarcoidosis, Langerhans cell histiocytosis (histiocytosis X), and lymphangioleiomyomatosis. Other genetic diseases associated with the development of pulmonary arterial hypertension include hereditary hemorrhagic telangiectasia and are addressed in another article in this issue. There are also the potential genetic contributions to our interactions with the environment such as high altitude and chronic hypoxemic lung diseases such as COPD and alveolar hypoventilation that may also predispose certain individuals to the development of pulmonary hypertension in these settings. The purpose of this review is to consider some of the potential genetic contributions that may underlay the development of pulmonary hypertension in association with other disorders. While some of these diseases can be defined by a single gene mutation, more frequently, associations between certain genes and susceptibility to pulmonary hypertension have been reported.

Hemoglobinopathies

The spectrum of known hemoglobinopathies associated with pulmonary hypertension is wide and may suggest a role of chronic hemolysis and/or splenectomy in the pathogenesis of pulmonary arterial hypertension. Indeed, many patients with inherited hemoglobin or red cell disorders are surgically or functionally asplenic, which may be a risk factor for the development of pulmonary arterial hypertension.[2]

Thalassemia

The thalassemias are a group of inherited disorders in hemoglobin characterized by hemolysis of varying severity. Alpha-thal is due to deletion of one of the alpha alleles of hemoglobin resulting in decreased alpha-chain synthesis. Alpha-thal is most common in persons from Asia and in the most severe form (missing all but one allele) the disease is called hemoglobin H (the hemoglobin formed when excess beta chains form tetramers). Beta-thalassemias are caused by point mutations and most frequently affect persons of Mediterranean descent. The genetic defect results in decreased transcription of the gene or premature chain termination. The net result is a decrease in beta-chain hemoglobin and increase in other hemoglobin forms including A and F. Additionally, the excess beta-chains precipitate in the red cell membrane leading to intramedullary and intravascular hemolysis.

Beta-thal is the most severe form of thalassemia but with a variable clinical course likely representing the severity of the underlying gene defect. Cardiomyopathy from iron overload as a result of repeated transfusions is the most common cardiopulmonary complication seen in patients with beta-thal. However, isolated pulmonary hypertension, not due to left ventricular dysfunction, is increasingly recognized in patients with beta-thal. While the numbers of patients who develop pulmonary hypertension is not clear, incidences ranging from 10% to 66% have been reported.[3,4] Factors associated with the development of pulmonary arterial hypertension in these patients include a history of splenectomy, thrombocytosis, severity of anemia, elevated nucleated red blood cells, hepatic cirrhosis, and iron overload. Some investigators have also reported an association with pulmonary thromboembolism.[5-9]

How these patients with thalassemia and pulmonary hypertension should be managed is not clear, as there have been no clinical trials performed in this patient population. General care includes maintaining near normal hematocrits, antiplatelet therapy (ie, aspirin), and consideration of anticoagulation.

Sickle cell anemia

Sickle cell anemia is the most common inherited hemoglobinopathy worldwide, affecting millions of individuals primarily of African descent. In the United States, the incidence is estimated at 1 in 400 to 600 African Americans. The molecular abnormality, a single amino acid substitution in the hemoglobin gene that is inherited in an autosomal recessive manner, results in the clinical syndrome of sickle crisis with microvascular occlusion and marked episodic intravascular hemolysis.

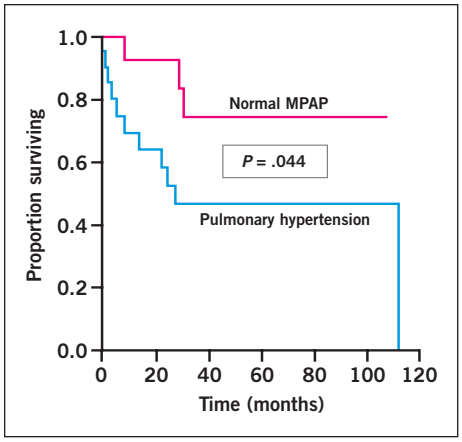
There is considerable variation in the clinical course between affected individuals. Chronic complications include avascular necrosis of long bones, iron overload states, thrombosis, chronic infections, cardiomyopathy, hepatic cir-

rhosis, and pulmonary hypertension. Factors associated with the development of pulmonary hypertension in patients with sickle cell anemia include older age, hypertension, ongoing intravascular hemolysis, and lower fetal hemoglobin concentrations.[10,11] The incidence of pulmonary hypertension is estimated between 20% and 40% of patients with sickle cell anemia and is associated with a higher likelihood of death (50% at 2 years, **Figure**).[10-12]

There is considerable debate as to whether pulmonary hypertension in sickle cell anemia is a primary pulmonary vascular process or a secondary process related to a number of complications of sickle cell anemia, including direct endothelial injury due to sickle cells, recurrent acute chest syndrome, chronic pulmonary infections, high-output states due to anemia thrombosis and, more recently described, decreased nitric oxide bioavailability due to scavenging by hemoglobin and superoxide.[13] Therefore, whether the development of pulmonary hypertension is a surrogate marker for advanced heart and lung disease resulting from the sequelae of sickle cell anemia or whether pulmonary hypertension per se is a risk factor for increased mortality is uncertain. It is evident that at least a significant percentage of patients included in studies of sickle cell anemia-associated pulmonary hypertension have evidence of elevated postcapillary pressure indicating that the pulmonary hypertension in these patients is a secondary, reactive process. Half of the patients with pulmonary hypertension in one study demonstrated an elevated pulmonary wedge pressure (PWP) as high as 28 mm Hg, whereas none of 14 patients without pulmonary hypertension had a PWP of >15.[12]

Pathologically, lung vessels demonstrate medial and intimal hypertrophy/hyperplasia, fibroelastic degradation of small arteries, arterioles, and venules, and thrombosis with recanalization. One autopsy study of 20 patients with sickle cell anemia reported that 60% of patients had evidence of plexiform lesions, a change characteristic of advanced pulmonary arterial hypertension, in lung sections.[14] While the pathogenesis of sickle cell anemia-associated pulmonary hypertension remains uncertain, and is likely multifactorial in most patients, greater numbers of patients and more detailed investigation are needed to accurately determine the role of pulmonary vascular disease in sickle cell anemia.

Treatment of patients with sickle cell anemia-associated pulmonary hypertension is also not clear. Supportive treatment is currently the mainstay of therapy for patients with sickle cell anemia, which includes folate supplementation, hydration, oxygen, transfusions, and measures aimed at increasing fetal hemoglobin levels (such as hydroxyurea). One report suggested that enhancing nitric oxide production with arginine supplementation may be helpful.[15]



Figure—Kaplan-Meier survival of patients with sickle cell anemia with and without pulmonary hypertension. The upper line is the survival estimate for sickle cell anemia patients without pulmonary hypertension, that is, with normal MPAP. The lower line is the survival estimate for sickle cell anemia patients with pulmonary hypertension, that is, with MPAP more than 25 mm Hg. The x-axis measures months of follow-up after cardiac catheterization. From: Castro O, Hoque M, Brown BD. Pulmonary hypertension in sickle cell disease: cardiac catheterization results and survival. *Blood*. 2003;101: 1257-1261. Copyright American Society of Hematology, used with permission.

Other red cell defects

Pulmonary arterial hypertension has also been associated with other, more rare defects in red cells. A common factor in these diseases is hemolytic anemia, as it is in the thalassemias and sickle cell anemia, and frequency of splenectomy, either surgical or due to splenic infarcts. Hereditary spherocytosis is due to an autosomal dominant defect in the red cell membrane protein spectrin that leads to a change in cell configuration from a bi-concave disc to a rounded phenotype. This in turn leads to poor distensability of the red cell and hemolysis primarily within the spleen. The disease presents with variable severity and has been associated with pulmonary arterial hypertension in a few individuals in whom other causes of pulmonary arterial hypertension have been excluded.[16]

Paroxysmal nocturnal hemoglobinuria is an acquired stem cell disorder. There is a genetic mutation leading to the inability of a group of cell surface proteins, complement-regulating surface proteins, to bind to cell membranes. The corresponding gene *PIGA* (phosphatidylinositol glycan class A) is on the X chromosome, and several mutations, from deletions to point mutations, have been reported. These changes increase the susceptibility of red cells to intravascular lysis by complement components. The disorder is manifest by episodic episodes of hemolysis, usually occurring at night, and can lead to severe anemia or pancytopenia. The syndrome is also associated with thrombosis, especially of the mesenteric and hepatic veins. The development of pulmonary arterial hypertension has been reported in association with paroxysmal nocturnal hemoglobinuria.[17]

Chronic Hypoxia

Hypoxia-induced pulmonary hypertension occurs in a wide variety of pulmonary diseases ranging from airways diseases, parenchymal lung diseases, high altitude exposure, and ventilatory control problems. While some of these diseases may have clearly defined genetic links (ie, alpha-1-antitrypsin caused emphysema), others may involve genes important in modifying the host response to chronic hypoxia (angiotensin converting enzyme and endothelial nitric oxide synthase gene polymorphisms, long-term population residence at high altitude, etc).

High-altitude exposure

Hints that moving to higher altitude may cause pulmonary hypertension come from both animal and population studies. In both situations, differences in genetic backgrounds and in physiologic adaptation to hypoxia are discernable. In the early 20th century, two Colorado veterinarians, George Glover and Isaac Newsom, reported the development of

"brisket disease" in cattle grazing on high Colorado plateaus. Brisket disease is edema of the dependent brisket and is associated with severe pulmonary hypertension and enlargement of the right ventricle. They noted that this disease was more common in cattle brought to the region for grazing than in the offspring of disease-free animals residing at high altitude.[18] In studies completed 50 years later using breeding of susceptible and resistant cattle, a genetic predisposition to the development of brisket disease was identified.[19-21] What this gene or genes are remains unknown.

The physiologic mechanism for this susceptibility to high altitude-induced pulmonary hypertension is, in part, related to an exaggerated acute hypoxic pulmonary pressor response. Interestingly, other mammals with a history of high altitude cultivation, including the llama and yak, which are resistant to the development of high altitude-induced pulmonary hypertension, have markedly blunted acute hypoxic pulmonary pressor responses.[22,23]

Increasingly, humans are extending their range of altitudes to include higher elevations for long-term residence. The development of pulmonary hypertension related to hypoxia varies considerably among distinct human populations living at high altitudes and may be related in part to the number of generations of high altitude living. Residents of Tibet, with the longest history of high altitude living, have a blunted hypoxic pulmonary pressor response compared to Andean Indians, who, in turn, have a blunted response compared to relative high altitude newcomers in the Rocky Mountains. Other physiologic differences more pronounced in Tibetans include blunted hypoxic ventilatory responses, higher lung diffusing capacities, higher hemoglobin concentrations, and slightly larger lungs, all suggesting long-term genetic adaptation to the environment. However, the nature of these genetic adaptations is largely unknown. Several hypotheses such as alterations in oxygen-sensing molecules, ion channel expression, redox states, and degree of muscularization of pulmonary vessels have been proposed.[22,23]

While the above discussion pertains to chronic exposure of populations at high-altitude, the acute adaptation to high altitude may also have a genetic component. Acute maladaptation to high altitude includes a spectrum of disease ranging from the relatively mild acute mountain sickness to more severe and life-threatening high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE). Several gene polymorphisms have been purported to be associated with the development of HAPE, including polymorphisms in the rennin-angiotensin system, endothelial nitric oxide synthase, and the major histocompatibility complex. Polymorphisms in the angiotensin converting enzyme have been associated with the development of chronic hypoxic pulmonary hypertension with the I/I genotype overrepresented[24,25] while other studies have failed to find a role of these polymorphisms in HAPE.[26,27] Polymorphisms in endothelial nitric oxide synthase and major histocompatibility complexes HLA-DR6 and -DQ4 are overrepresented in persons who develop HAPE.[28,29] Interestingly, the HLA-DR6 polymorphisms are also associated with hypoxic pulmonary hypertension.[29] While these associations are interesting, unfortunately they only begin to hint at the possible mechanisms for genetic susceptibility to hypoxia-induced pulmonary vascular disease.

How chronic exposure to higher altitude should be taken in the context of a diagnosis of pulmonary hypertension must be determined on a case-by-case basis. Many pulmonary hypertension specialists recommend that patients living at higher altitudes consider relocating to remove this as a confounding factor.

Hypoxia due to lung disease

Chronic hypoxia is a consequence of many different lung diseases and is thought to underlie the development of pulmonary hypertension in some of these patients. However, not all patients with hypoxemic lung disease develop pulmonary hypertension. Pulmonary hypertension in association with diseases such as chronic obstructive pulmonary disease (COPD) is therefore likely associated with other modifying conditions, which might be genetic in nature. The development of pulmonary hypertension in association with COPD can be clinically variable and carries a poor prognosis. Several genetic modifiers have been proposed to contribute to the development of pulmonary hypertension in some patients with COPD.

Involvement of the serotonin transporter has been implicated in the development of pulmonary hypertension in patients with COPD (see article by Eddahibi and Adnot in this issue).[30] The BB genotype of a polymorphism in intron 2 of the endothelial nitric oxide synthase gene has also been associated with more severe pulmonary hypertension in COPD patients compared to the AA or AB genotypes.[31]

Polymorphisms in the angiotensin converting enzyme gene have also been implicated in some studies of COPD-associated pulmonary hypertension. However, as opposed to chronic hypoxia-induced pulmonary hypertension, where the II genotype is overrepresented, the DD genotype appears to correlate with more severe COPD-associated pulmonary hypertension.[32] Patients with the DD genotype demonstrated more exercise impairment and higher pulmonary arterial pressures and were less likely to improve with treatment with angiotensin converting enzyme inhibitors or with oxygen.[33-36]

While these observations may suggest potential mechanisms for the apparent variable course of patients with similar impairment of lung function, to date clinical treatment decisions based on these genotypes are not available. At present, for COPD-associated pulmonary hypertension, cessation of smoking, treatment of airway obstruction, and supplemental oxygen remain the mainstays of therapy for all patients.

Hypoxia due to disorders of ventilation

Impaired ventilatory control can lead to alveolar hypoxemia and pulmonary hypertension. The most common situation in which this occurs is in nocturnal hypoventilation associated with sleep apnea. However, hypoventilation syndromes can also occur during waking hours and may be associated with pulmonary hypertension.

Sleep apnea is common and is frequently found in families. While this may be associated with the presence of obesity, which also occurs more frequently in families, there may be a genetic predisposition to sleep apnea. Recently, a

whole genome scan identified a susceptibility loci in a region on chromosome 8q that was associated with increased apnea-hypopnea indexes in an African American family cohort.[37] Others have also identified polymorphisms in the haptoglobin gene and found that patients with the 2-2 genotype had a 2.3-fold risk of cardiovascular complications of sleep apnea compared to patients with the 2-1 genotype.[38] Thus, genetic modifiers may play a role not only in the development of sleep apnea, but also in the complications of sleep apnea. To date, potential associations with, or modifiers of, the development of pulmonary hypertension in association with sleep apnea have not been identified.

Congenital central hypoventilation syndrome, also known as Ondine's curse, is associated with mutations in the PHOX2B gene family. Mutations in this gene lead to impaired noradrenergic neuronal development.[39] This results in significant central hypoventilation as well as other abnormalities of autonomic nervous innervation, in particular, Hirschsprung disease of the intestine. The syndrome is frequently associated with significant cor pulmonale likely due to severe alveolar hypoxemia.

Metabolic Diseases

The presence of pulmonary arterial hypertension in association with rare genetic metabolic diseases suggests the possibility that the mechanisms for the development of pulmonary arterial hypertension may include other previously unknown metabolic pathways or alternative effects of known genetic mutations.

Gaucher disease

Gaucher disease is a lysosomal storage disease due to inherited deficiency of glucocerbrosidase enzyme activity leading to accumulation of lipid-laden cells (Gaucher cells) in multiple organs including the central nervous system, spleen, liver, lymph, and lung. It is an autosomal recessive disease with variable clinical expression thought in part to be due to genetic heterogeneity of the mutations with the L444P mutation most commonly associated with pulmonary complications[40] and non-N370S mutations associated with the development of pulmonary arterial hypertension.[41] Pulmonary arterial hypertension in Gaucher disease may be related to direct pulmonary vascular involvement with Gaucher cells[42] as well as chronic hepatic dysfunction and portal hypertension. Splenectomy is also associated with pulmonary arterial hypertension in these patients.[41,43] Interestingly, the presence of the ACE I allele is associated with pulmonary arterial hypertension in these patients as well.[41] Enzyme replacement therapy has been reported to decrease rates of disease and has been associated with regression of pulmonary arterial hypertension.[41]

Other Metabolic Diseases

Type Ia glycogen storage disease has been reported in association with pulmonary arterial hypertension.[44] It is an autosomal recessive disorder leading to deficiency of the glucose-6-phosphatase enzyme. It is associated with dyslipidemias and hepatic steatosis, hepatic adenomas, and increased rates of vascular thrombosis. While the occurrence of pulmonary arterial hypertension in these patients is rare, it may be related to the presence of hepatic dysfunction, portosystemic shunts, and in a recent report, markedly increased levels of plasma serotonin.[44] Unfortunately, in the patients with type-1a glycogen storage disease and pulmonary arterial hypertension, rapid progression of right heart with failure and death has been observed.

Serotonin may also play a role in the report of pulmonary arterial hypertension in a patient with familial platelet storage pool disease. This disease is an autosomal dominant, rare disease characterized by normal platelet counts but impaired platelet aggregation and decreased numbers of dense granules. This leads to decreased platelet, but increased plasma, serotonin levels among other defects. In a single patient with pulmonary arterial hypertension and familial platelet storage pool disease, treatments with a serotonin receptor antagonist, ketanserin, improved his pulmonary vascular resistance.[45]

Summary

The genetic contributions that may hint at the pathogenesis of pulmonary hypertension are expanding at a rapid pace. While in some instances, such as familial pulmonary arterial hypertension or in HHT-related pulmonary arterial hypertension, a specific gene has been identified, we are also beginning to recognize the important contributions that other modifier genes may have in susceptible individuals (ie, angiotensin converting enzyme, endothelial nitric oxide synthase, etc). We are also beginning to expand our hypotheses regarding the pathogenesis of pulmonary hypertension by considering the metabolic and physiologic derangements that characterize other genetic disorders that are associated with its development (ie, hemoglobin disorders, metabolic disease). Pharmacogenomic studies, wherein a patient is characterized both clinically and genetically in order to identify the treatment most likely to be of benefit to that particular individual, are increasingly being conducted. Unfortunately, we are still a long way from this type of directed therapy; however, each new genetic and clinical association brings us closer to this goal.

References

1. Simonneau G, Galie N, Rubin LJ, Langleben D, Seeger W, Domenighetti G, Gibbs S, Lebrec D, Speich R, Beghetti M, Rich S, Fishman A. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol*. 2004;43:5S-12S.
2. Hoeper MM, Niedermeyer J, Hoffmeyer F, Flemming P, Fabel H. Pulmonary hypertension after splenectomy? *Ann Intern Med.* 1999;130:506-509.
3. Derchi G, Fonti A, Forni GL, Galliera EO, Cappellini MD, Turati F, Policlinico OM. Pulmonary hypertension in patients with thalassemia major. *Am Heart J.* 1999;38:384.
4. Du ZD, Roguin N, Milgram E, Saab K, Koren A. Pulmonary hypertension in patients with thalassemia major. *Am Heart J.* 1997;134:532-537.
5. Aessopos A, Farmakis D, Karagiorga M, Voskaridou E, Loutradi A, Hatziliami A, Joussef J, Rombos J, Loukopoulos D. Cardiac involvement in thalassemia intermedia: a multicenter study. *Blood*. 2001;97:3411-3416.
6. Atichartakarn V, Likittanasombat K, Chuncharunee S, Chanda namattha P, Worapongpaiboon S, Angchaisuksiri P, Aryurachai K. Pulmonary arterial hypertension in previously splenectomized patients with beta-thalassemic disorders. *Int J Hematol.* 2003;78:139-145.

7. Hahalis G, Manolis AS, Apostolopoulos D, Alexopoulos D, Vagenakis AG, Zoumbos NC. Right ventricular cardiomyopathy in beta-thalassaemia major. *Eur Heart J.* 2002;23:147-156.
8. Taher A, Abou-Mourad Y, Abchee A, Zalouaa P, Shamseddine A. Pulmonary thromboembolism in beta-thalassemia intermedia: are we aware of this complication? *Hemoglobin.* 2002;26:107-112.
9. Wu KH, Chang JS, Su BH, Peng CT. Tricuspid regurgitation in patients with beta-thalassemia major. *Ann Hematol.* 2004;83:779-783.
10. Ataga KI, Sood N, De Gent G, Kelly E, Henderson AG, Jones S, Strayhorn D, Lail A, Lieff S, Orringer EP. Pulmonary hypertension in sickle cell disease. *Am J Med.* 2004;117:665-669.
11. Gladwin MT, Sachdev V, Jison ML, Shizukuda Y, Plehn JF, Minter K, Brown B, Coles WA, Nichols JS, Ernst I, Hunter LA, Blackwelder WC, Schechter AN, Rodgers GP, Castro O, Ognibene FP. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. *N Engl J Med.* 2004;350:886-895.
12. Castro O, Hoque M, Brown BD. Pulmonary hypertension in sickle cell disease: cardiac catheterization results and survival. *Blood.* 2003;101:1257-1261.
13. Aslan M, Freeman BA. Oxidant-mediated impairment of nitric oxide signaling in sickle cell disease—mechanisms and consequences. *Cell Mol Biol (Noisy-le-grand).* 2004;50: 95-105.
14. Haque AK, Gokhale S, Rampy BA, Adegboyega P, Duarte A, Saldana MJ. Pulmonary hypertension in sickle cell hemoglobinopathy: a clinicopathologic study of 20 cases. *Hum Pathol.* 2002;33:1037-1043.
15. Morris CR, Morris SM, Jr., Hagar W, Van Warmerdam J, Claster S, Kepka-Lenhart D, Machado L, Kuypers FA, Vichinsky EP. Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease? *Am J Respir Crit Care Med.* 2003;168:63-69.
16. Hayag-Barin JE, Smith RE, Tucker FC Jr. Hereditary spherocytosis, thrombocytosis, and chronic pulmonary emboli: a case report and review of the literature. *Am J Hematol.* 1998;57:82-84.
17. Heller PG, Grinberg AR, Lencioni M, Molina MM, Roncoroni AJ. Pulmonary hypertension in paroxysmal nocturnal hemoglobinuria. *Chest.* 1992;102:642-643.
18. Glover GH, Newsome IE. Brisket disease (dropsy of high altitudes). Colorado Agriculture Experimental Station Bulletin No. 204, 1915.
19. Weir EK, Tucker A, Reeves JT, Will DH, Grover RF. The genetic factor influencing pulmonary hypertension in cattle at high altitude. *Cardiovasc Res.* 1974;8:745-749.
20. Will DH, Hicks JL, Card CS, Alexander AF. Inherited susceptibility of cattle to high-altitude pulmonary hypertension. *J Appl Physiol.* 1975;38:491-494.
21. Will DH, Hicks JL, Card CS, Reeves JT, Alexander AF. Correlation of acute with chronic hypoxic pulmonary hypertension in cattle. *J Appl Physiol* 1975;38:495-498.
22. Fagan KA, Weil JV. Potential genetic contributions to control of the pulmonary circulation and ventilation at high altitude. *High Alt Med Biol.* 2001;2:165-171.
23. Ward MP, Milledge JS, West JB. *High Altitude Medicine and Physiology.* London: Arnold; 2000.
24. Aldashev AA, Sarybaev AS, Sydykov AS, Kalmyrzaev BB, Kim EV, Mamanova LB, Maripov R, Kojonazarov BK, Mirrakhimov MM, Wilkins MR, Morrell NW. Characterization of high-altitude pulmonary hypertension in the Kyrgyz: association with angiotensin-converting enzyme genotype. *Am J Respir Crit Care Med.* 2002;166:1396-1402.
25. Morrell NW, Sarybaev AS, Alikhan A, Mirrakhimov MM, Aldashev AA. ACE genotype and risk of high altitude pulmonary hypertension in Kyrghyz highlanders. *Lancet.* 1999;353:814.
26. Dehnert C, Weymann J, Montgomery HE, Woods D, Maggiorini M, Scherrer U, Gibbs JS, Bartsch P. No association between high-altitude tolerance and the ACE I/D gene polymorphism. *Med Sci Sports Exerc.* 2002;34:1928-1933.
27. Hotta J, Hanaoka M, Droma Y, Katsuyama Y, Ota M, Kobayashi T. Polymorphisms of renin-angiotensin system genes with high-altitude pulmonary edema in Japanese subjects. *Chest.* 2004;126:825-830.
28. Droma Y, Hanaoka M, Ota M, Katsuyama Y, Koizumi T, Fujimoto K, Kobayashi T, Kubo K. Positive association of the endothelial nitric oxide synthase gene polymorphisms with high-altitude pulmonary edema. *Circulation.* 2002;106:826-830.
29. Hanaoka M, Kubo K, Yamazaki Y, Miyahara T, Matsuzawa Y, Kobayashi T, Sekiguchi M, Ota M, Watanabe H. Association of high-altitude pulmonary edema with the major histocompatibility complex. *Circulation.* 1998;97:1124-1128.
30. Eddahibi S, Chaouat A, Morrell N, Fadel E, Fuhrman C, Bugnet AS, Dartevelle P, Housset B, Hamon M, Weitzenblum E, Adnot S. Polymorphism of the serotonin transporter gene and pulmonary hypertension in chronic obstructive pulmonary disease. *Circulation.* 2003;108:1839-1844.
31. Yildiz P, Oflaz H, Cine N, Erginel-Unaltuna N, Erzengin F, Yilmaz V. Gene polymorphisms of endothelial nitric oxide synthase enzyme associated with pulmonary hypertension in patients with COPD. *Respir Med.* 2003;97:1282-1288.
32. van Suylen RJ, Wouters EF, Pennings HJ, Cheriex EC, van Pol PE, Ambergen AW, Vermelis AM, Daemen MJ. The DD genotype of the angiotensin converting enzyme gene is negatively associated with right ventricular hypertrophy in male patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 1999;159:1791-1795.
33. Kanazawa H, Hirata K, Yoshikawa J. Effects of captopril administration on pulmonary haemodynamics and tissue oxygenation during exercise in ACE gene subtypes in patients with COPD: a preliminary study. *Thorax.* 2003;58:629-631.
34. Kanazawa H, Hirata K, Yoshikawa J. Influence of oxygen administration on pulmonary haemodynamics and tissue oxygenation during exercise in COPD patients with different ACE genotypes. *Clin Physiol Funct Imaging.* 2003;23:332-336.
35. Kanazawa H, Okamoto T, Hirata K, Yoshikawa J. Deletion polymorphisms in the angiotensin converting enzyme gene are associated with pulmonary hypertension evoked by exercise challenge in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2000;162:1235-1238.
36. Kanazawa H, Otsuka T, Hirata K, Yoshikawa J. Association between the angiotensin-converting enzyme gene polymorphisms and tissue oxygenation during exercise in patients with COPD. *Chest.* 2002;121:697-701.
37. Palmer LJ, Buxbaum SG, Larkin EK, Patel SR, Elston RC, Tishler PV, Redline S. Whole genome scan for obstructive sleep apnea and obesity in African-American families. *Am J Respir Crit Care Med.* 2004;169:1314-1321.
38. Lavie L, Lotan R, Hochberg I, Herer P, Lavie P, Levy AP. Haptoglobin polymorphism is a risk factor for cardiovascular disease in patients with obstructive sleep apnea syndrome. *Sleep.* 2003;26:592-595.
39. Amiel J, Laudier B, Attie-Bitach T, Trang H, de Pontual L, Gener B, Trochet D, Etchevers H, Ray P, Simonneau M, Vekemans M, Munnich A, Gaultier C, Lyonnet S. Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. *Nat Genet.* 2003;33:459-461.
40. Santamaria F, Parenti G, Guidi G, Filocamo M, Strisciuglio P, Grillo G, Farina V, Sarnelli P, Rizzolo MG, Rotondo A, Andria G. Pulmonary manifestations of Gaucher disease: an increased risk for L444P homozygotes? *Am J Respir Crit Care Med.* 1998;57:985-989
41. Mistry PK, Sirrs S, Chan A, Pritzker MR, Duffy TP, Grace ME, Meeker DP, Goldman ME. Pulmonary hypertension in type 1 Gaucher's disease: genetic and epigenetic determinants of phenotype and response to therapy. *Mol Genet Metab.* 2002;77:91-98.
42. Ross DJ, Spira S, Buchbinder NA. Gaucher cells in pulmonary-capillary blood in association with pulmonary hypertension. *N Engl J Med.* 1997;336:379-381.
43. Elstein D, Klutstein MW, Lahad A, Abrahamov A, Hadas-Halpern I, Zimran A. Echocardiographic assessment of pulmonary hypertension in Gaucher's disease. *Lancet.* 1998;351:544-1546.
44 Humbert M, Labrune P, Sitbon O, Le Gall C, Callebert J, Herve P, Samuel D, Machado R, Trembath R, Drouet L, Launay JM, Simonneau G. Pulmonary arterial hypertension and type-I glycogen-storage disease: the serotonin hypothesis. *Eur Respir J.* 2002;20:59-65.
45. Herve P, Drouet L, Dosquet C, Launay JM, Rain B, Simonneau G, Caen J, Duroux P. Primary pulmonary hypertension in a patient with a familial platelet storage pool disease: role of serotonin. *Am J Med.* 1990;89:117-120.