Saudi Guidelines on the Diagnosis and Treatment of Pulmonary Hypertension: Genetics of pulmonary hypertension

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Abstract:
 Pulmonary hypertension (PH) is a phenotype characterized by functional and structural changes in the pulmonary vasculature, leading to increased vascular resistance. The World Health Organization has classified PH into five different types: arterial, venous, hypoxic, thromboembolic or miscellaneous; details are available in the main guidelines. Group I of this classification, designated as pulmonary arterial hypertension (PAH), will remain the main focus here. The pathophysiology involves signaling, endothelial dysfunction, activation of fibroblasts and smooth muscle cells, interaction between cells within the vascular wall, and the circulating cells; as a consequence, plexiform lesions are formed, which is common to both idiopathic and heritable PAH but are also seen in other forms of PAH. As the pathology of PAH in the lung is well known, this article focuses on the genetic aspects associated with the disease and is a gist of several available articles in literature.

Key words:
 Bone morphogenetic protein receptor type II, transforming growth factors, activin receptor-like kinase 1, endoglin, genetics, pulmonary hypertension

Pulmonary hypertension (PH) is a phenotype characterized by functional and structural changes in the pulmonary vasculature, leading to increased vascular resistance. The World Health Organization has classified PH into five different types: Arterial, venous, hypoxic, thromboembolic or miscellaneous; details are available in the main guidelines. Group I of this classification, designated as pulmonary arterial hypertension (PAH), will remain the main focus here. The pathophysiology involves signaling, endothelial dysfunction, activation of fibroblasts and smooth muscle cells, interaction between cells within the vascular wall, and the circulating cells; as a consequence, plexiform lesions are formed, which is common to both idiopathic PAH (IPAH) and heritable PAH (HPAH), but are also seen in other forms of PAH.

There have been major advances in understanding the physiological pathways associated with PAH. An array of vasoactive compounds, growth factors and inflammatory markers are involved in enhancing or reducing the risk of disease occurrence, promoting or reorganizing the pulmonary vascular changes, amply pointing to the fact that the phenotype is multigenic and multifactorial. Several of these molecules are involved in more than one function and helped greatly in anticipating new potential therapeutic targets. As the pathology of PAH in the lung is well known, this article focuses on the genetic aspects associated with the disease and is a gist of several available articles in the literature.

Genetic of Pulmonary Arterial Hypertension

Last decade has provided substantial knowledge on the genetic basis of PAH. Investigators used molecular approaches such as the candidate gene and genome-wide association to identify the variants in polymorphisms on genes or chromosomes. Several candidate genes of various physiological pathways have shown potential to be candidate markers for PAH; among these, the receptor members of the TGF-β superfamily have emerged as the leading causal markers of HPAH. In addition to the polymorphisms, numerous mutations have been identified in the...
genes of this family, foremost among them are heterozygous mutations of the BMPRII gene. Nearly 70% of HPAH patients and 10-20% of IPAH patients show involvement of BMPRII mutations. Compared with this, fewer mutations have been reported in TGF-\(\beta\), Smads and ALK-1. It is pertinent to differentiate here between a polymorphism and a mutation in a genome; compared to the former the latter is rare in frequency in a population.

Bone morphogenetic protein receptor type II emerges as a major contributor to PAH pathology. The gene seems highly vulnerable to the intrusive environmental changes around a target location leading to mutations in susceptible subjects, which may even be transferred to off-springs. However, importantly, emergence of mutations in select patients of PAH, especially the HPAH, emphasizes the involvement of polymorphisms or mutations of additional genes in an epistatic mode.

The status of polymorphisms and mutations in pulmonary arterial hypertension

It will take some time to provide the exact number of mutations and polymorphisms in any candidate gene that associate with PAH; however, literature suggests BMPRII to be the gene of preference with more than 300 variants identified in hereditary and idiopathic patients, but with no demarcation for the two types of PAH. BMPRII is located on chromosome 2q33 with 13 exons. The gene has four well-characterized domains namely the extracellular, transmembrane, kinetic, and cellular that define the three-dimensional structure and function of the receptor protein. The causal effect is supposedly coming from four major types of mutations, the nonsense, frame-shift, splice-site, and gene duplications or deletions. In addition, a two-base consecutive substitution of GC to AT has also been observed. The nonsense-mediated decay provides evidence of haploinsufficiency and the missense mutations of heterogeneous functional defects in BMPRII activity.

The extracellular and kinase domain mutations contribute much of the functional changes in the receptor protein. The extracellular domain constitutes the 10-cystein residues, which form the disulfide bridges to provide stability. Mutations at these cysteine sites are known to disrupt the structural integrity and the translocation. The kinetic domain understandably is the functional entity and the missense mutations in this region amount to disrupting the substrate binding property. One of the widely believed causes of these mutations is the premature truncation. This domain is comprised of 12 subdomains and caters to the important process of phosphorylation and energy transfer. Mutations in this region thus impede the receptor integrity and thereby impede the signaling process. Furthermore, phosphorylation is an important step that activates and inactivates several of the kinases during signaling process. However, as the cascading process is complicated, several molecules no doubt contribute to the pathophysiology.

Among the other constituents of TGF-\(\beta\) signaling, Smad4 is vital to both the TGF-\(\beta\) and BMPRII translocation to the nucleus. The
Smad4 amino-terminal domain has two missense mutations, L43S and R100T, causing structural changes to the protein that results in dependent proteins not efficiently translocated to the nucleus and thus, produce severely defective transcriptional response. Of note, the ALK-1 mutations are seen in a minority of patients with hereditary hemorrhagic telangiectasia associated with PAH.

Apart from the signaling pathway, none of the other pathways are studied in so detail, however as they are vital to the pathophysiology of PAH, few of them deserve description for genetic contribution [Table 1].

Endothelin-1, a potent vasoconstrictor, has been implicated in the pathogenesis of PAH; it brings out opposite effects when interacts with its two receptors, ETA and ETB. ET-I polymorphisms, viz., −3A/4A, (CT) n–(CA) n repeat, G2288T, and G594T (Lys198Asn) were found to associate with high-altitude pulmonary edema (HAPE), IPAH and chronic obstructive pulmonary disease (COPD). Interestingly, a crosstalk between these polymorphisms and the insertion/deletion polymorphism of another potent vasoconstrictor gene, angiotensin-I converting enzyme (ACE) was observed in the HAPE patients. Polymorphisms in the ETA and ETB receptor genes also have been identified.

Thromboxane synthase (TBXAS1), a cytochrome P450 enzyme, converts prostaglandin H2 into TXA2, which is a potent vasoconstrictor. TXA2 induces platelet aggregation, smooth muscle contraction, and regulates blood flow; may promote mitogenesis and apoptosis. In the vasculature, TXA2 is released from platelets, whereas prostacyclin twelve (PGI2) is released from endothelial cells.

12 coding-region variants have been identified in the human TBXAS1 gene of which three nonsynonymous polymorphisms, 772A > G (Lys258Glu), 1348G > A (Glu450 Lys), and 1352C > A (Thr451Asn) showed higher likelihood to affect protein function. PGI2, an effective vasodilator, inhibits platelet activation to prevent the platelet plug formation. Together, TXA2 and PGI2 play a role in the maintenance of hemostasis. Although, polymorphisms have been recognized in the prostacyclin synthase gene in relation to chronic thromboembolic PH and cardiovascular function; however, the gene has yet to be worked thoroughly to establish its role.

Table 1: The potential candidate genes for pulmonary arterial hypertension

| Gene name* | Chromosome position | Size | Exons | SNPs/mutations | Function |
|------------|---------------------|------|-------|----------------|----------|
| BMPRII     | 2; location: 2q33-q34 | 191.42 kb | 13 | >300 mutations | Vasoconstriction |
| ALK-1      | 12; location: 12q11-q14 | 16.45 kb | 11 | >270 mutations in HHT related hypertension | Disrupts pulmonary vasculature |
| TGF-β1     | 19; location: 19q13.1 | 23.0 kb | 7 | Several | Vasoconstriction |
| 5-HTT      | 17; location: 17q11.2 | 41.6 kb | 14 | 5HTTLPR I/D rs25531 rs2066713 rs1042173 | Vasoconstriction |
| ET-1       | 6; location: 6p24.1 | 8.6 kb | 5 | (CT) n–(CA) n repeat −3A/4A G2288T G594T | Vasoconstrictor and cell proliferation |
| APLN       | X; location: Xq25 | 11.5 kb | 3 | rs3115757 rs3761581 rs2235306 rs3761581 T-1860C | Vasodilatation |
| TBXAS1     | 7; location: 7q34-q35 | 243.2 kb | 25 | A772G G1348A C1352A | Vasoconstrictor |
| NOS3       | 7; location: 7q36 | 23.5 kb | 27 | G894T 4b/4a T-786C rs6090996 rs6091000 C1117A | Produces NO, a vasodilator |
| PTGIS      | 20; location: 20q13.13 | 64.30 kb | 9 | rs8090996 rs6091000 C1117A | Hemostasis |
| VIP        | 6; location: 6q25 | 8.96 kb | 7 | rs555985 | Vasodilatation |
| ANP/NPPA   | 1; location: 1p36.21 | 2.63 kb | 4 | C-664G G1837A T2238C | Vasodilatation |
| KCNA5      | 12; location: 12p13 | 2.865 kb | 1 | G773A G861C | Smooth muscle contraction |
| ACE        | 17; location: 17q23.3 | 44.7 kb | 44 | ACE I/D | Vasoconstriction |

*Citation are not provided, BMPRII = Bone morphogenetic protein receptor type II, ALK-1 = Activin receptor-like kinase 1, ET-1 = Endothelin-1, TBXAS1 = Thromboxane synthase, NOS3 = Nitric oxide synthase, VIP = Vasoactive intestinal peptide, ANP = Atrial natriuretic peptide, NNPA = Natriuretic peptide precursor A, ACE = Angiotensin-I converting enzyme, TGF-β1 = Transforming growth factor-beta, PTGIS = Prostacyclin synthase, NO = Nitric oxide, 5-HTT = 5-hydroxytryptamine transporter, SNPs = Single nucleotide polymorphisms, APLNR = Apelin receptor.
Nitric oxide synthase 3 polymorphisms, especially the −786T/C, 4b/4a and 894G>T (Glu298Asp), have been implicated in cardiovascular and pulmonary diseases including PAH and HAPE. Further, the 4b/4a variable number of tandem repeat has been reported to work as a small RNA, thereby regulating the function of the gene. Mutant alleles of these polymorphisms associate with decreased NO level.[16] NO is a multifunction molecule; hence, the low levels in the circulation may impede several of the physiological functions. Furthermore, a crosstalk between the polymorphisms of NOS3 with ET-I and NOS3 with ACE has been reported that makes essence of epistasis and thus the combinations and the respective biochemical levels vital to the vascular homeostasis.

Genetic variations in the Kv1.5 channel gene (KCNA5), such as the two nonsynonymous mutations, G773A (G182R) and G861C (E211D) that localize to the highly conserved NH2-terminal tetramerization domain (T1) have been identified in IPAH patients.[17] T1 is important for proper channel assembly, association with regulatory Kv beta-subunits and localization of the channel to the plasma membrane. Likewise, a correlation was observed between alleles of the T937A and G2870A polymorphisms and NO level and anorexigen fenfluramine (an appetite suppressant that increases the risk of PAH) in IPAH patients.

The correlation of polymorphisms with NO level also suggest of an interaction between KCNA5 and NOS3. A recent study reported a novel heterozygous missense variant c.608 G→A (G203D) with additional five similar variants in another member KCNK3 in hereditary and idiopathic patients.[18] Variants in these genes may predispose individuals to high risk for conditions and diseases related to dysfunctional Kv channels.

The serotonin transporter (SERT) gene, SERT has been studied in IPAH without any association. However, the gene has been strongly associated with hypoxic PH in COPD.[19] Because, 5-HT is a strong vasoconstrictor and mitogen, its role in PAH cannot be overlooked. An insertion/deletion polymorphism in the promoter region of the SERT gene with higher frequency of the long allele, elevated gene expression and function has been associated with COPD.

Relevance of interactions among genes
A physiological function is an outcome of a metabolism or pathway and in many cases it involves more than one pathway indicating clearly the interaction of several molecules in bringing out that function; hence, interactions (epistasis) between and among the relevant genes is obvious. Studying genetic interactions can reveal the nature and strength of mutations, functional redundancy, gene function and thus the protein interactions so as to unveil the physiological or pathophysiological function. Because protein complexes are responsible for most biological functions, identification of genetic interactions is an obvious choice. Epistasis thus becomes a powerful tool in defining the effects of one gene as influenced by one or several other genes, which are sometimes called modifier genes or vice versa. The crosstalk between several genes has been observed in relation to hypoxic diseases including COPD and PAH as has been discussed elsewhere in the text. The Figure 1 depicts the interactions of signaling pathway, vasoactive pathway, oxidative stress, growth factors, and serotonin pathway.

Genetic testing in diagnosis and prognosis
Genetic testing, in a disease or heritable disease, is primarily the analysis of chromosomes or DNA or candidate genes that would reflect on the respective gene products as proteins/enzymes and other respective metabolites so as to detect genotypes, mutations, and correlations. Specific methods to screen patients for genotypes (polymorphism), mutations and gene rearrangements include sequencing, deep-sequencing, melting curve analysis, denaturing high-performance liquid chromatography, and multiplex ligation-dependent probe amplification.

Sequencing seems the ideal option because of the sporadic distribution of mutations in the patients. Whole genome sequence will not serve the purpose, as of now. Presently, as knowledge is available only for the BMPRII gene, sequencing of this gene or alternatively the exons and the splice junctions may be introduced as a routine diagnostic test and may be extended to TGF-β, endoglin, ALK-1 and other genes, but depending upon the type of disease in relation to PAH. A dedicated customized chip having all the polymorphisms and mutations of identified candidate genes for PAH is an alternative to screen a genome for susceptible variants.

Any individual with a family history of PAH or IPAH, without other known affected family members, may be advised the test. Revelation of an individual’s genetic set up may have unsavory psychological and social repercussions and surely will require counseling, but it may immensely benefit in predisposition.[20] It may go a long way in identifying the susceptible cases and to take timely medical advice and intervention and will surely help in family planning. Equally important, the test will bring in lifelong contentment to those family members who are not at risk of the disease. Thus, the mutant carrier or noncarrier both would benefit from this testing. The cost of each test could be a matter of concern if the test is rare; presently it is available in few countries. Precautionary screening may be performed at any stage starting from prenatal to adulthood. With the fast emerging concept of personalized medicine or pharmacogenomics, the genetic screening would be a routine test. Regulations need to be drawn to protect the interest of patients or the susceptible individuals.

Among all the candidate genes discussed here, screening of the BMPRII mutations seems the best choice for initial diagnosis; however, few pressing issues make it a vulnerable choice: Foremost only 70% of HPAH patients and nearly 20% of IPAH patients show association — which means involvement of other genes need to be worked out. Such a practice would certainly help in identifying the causal markers and thus the physiological mechanisms that act differentially under the changed genetic environment. It will also help resolve the clinical severity and the subsequent therapeutic steps and follow-ups.

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