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Genetic and genomic approaches to pulmonary vascular diseases

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Abstract

Pulmonary vascular diseases include pulmonary arterial hypertension, pulmonary venous hypertension, pulmonary embolism and chronic thromboembolic disease. The mutations of *BMPR2* were identified as major causes of primary pulmonary hypertension. The Factor V Leiden mutation is the most common genetic risk for pulmonary embolism. Candidate gene studies, genome-wide association studies, epigenetic studies, transcriptional profiling, miRNA profiling also identified novel causes for the diseases. Genetic and genomic approaches for pulmonary vascular disease provided new insights of the mechanisms and revealed the novel therapeutic targets. In this review, we summarise the current progress of genetic and genomic approaches for pulmonary vascular diseases and also discuss the future directions of the research for the diseases.

Introduction

Pulmonary vascular diseases refer any abnormal condition that affects the blood vessels along the route between the lungs and heart. There are two main kinds of pulmonary vascular diseases: pulmonary hypertension and pulmonary embolism. Pulmonary hypertension includes pulmonary arterial hypertension (PAH) and pulmonary venous hypertension. PAH is a rare, severe disease resulting from progressive obliteration of small pulmonary arteries by proliferating vascular cells. The prevalence of PAH is less than 50 cases per million in the population [1]. However, it is substantially higher at certain risk groups. For example, the prevalence is 0.5% in HIV-infected patients [2], 7-27% in patients with systemic sclerosis [3-5] and 2-3.75% in patients with sickle cell disease [6,7]. Idiopathic PAH (IPAH) has an annual incidence of 1-2 cases per million people in the US and Europe and is 2-4 times as common in women as in men [8,9]. The average age at diagnosis for IPAH is around 45 years, although the onset of symptoms can occur at any age [10]. It is likely that IPAH accounts for at least 40% of cases of PAH, with associated pulmonary arterial hypertension (APAH) accounting for the majority of the remaining cases [11]. PAH associated with the new-born is known as persistent pulmonary hypertension of the new-born (PPHN) and has been estimated to occur in 0.2% of live-born term infants. Pulmonary hypertension complicates the course of more than 10% of all neonates with respiratory failure [12].

Both pulmonary embolism (PE) and deep venous thrombosis (DVT) are known as venous thromboembolism (VTE). VTE incidence among whites of European origin exceeds 1 per 1000, one-quarter of patients present as sudden death. Of those patients who survived, 30% developed VTE recurrence and venous stasis syndrome within 10 and 20 years [13]. Clinically, pulmonary embolism (PE) and deep venous thrombosis (DVT) are often regarded as two aspects of the same disease. There were evidence that 50-70% of patients with DVT have silent PE and DVT is present in 70–90% of patients with PE [14]. Clinical reports of PE are sharply increased yearly in China and other Asia countries [15]. There are nearly 900,000 new PE cases in USA every year [16]. PE is the third leading cause of death only lags behind malignancy and myocardial infarction [17]. Pulmonary embolism in rare instance can develop to chronic thromboembolic disease if pulmonary embolism is not reabsorbed by the body. It could develop as chronic thromboembolic pulmonary hypertension [18].

In recent years, with the development of genetic and genomic approaches, many genetic factors that influence the mechanisms of pulmonary vascular diseases have been identified. The systemic approaches with linkage studies, genome-wide association studies, epigenetic studies, RNA sequencing, miRNA sequencing and chromatin immunoprecipitation (ChIP) sequencing brought a lot of information to pulmonary vascular diseases. In this review, we briefly introduce current methodologies for genetic and genomic approaches to complicated diseases and summarize the progress for the pulmonary vascular diseases mainly for pulmonary hypertension and pulmonary embolism; we also discuss the future research directions of the diseases.

The methods for genetic and genomic approaches to complicated diseases

The genetic approaches to complicated diseases such as pulmonary vascular diseases include candidate gene studies, positional cloning studies and genome-wide association studies (GWASs). Candidate gene study needs to have relatively big case and control groups

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to increase the power for statistical analysis. Positional cloning is another genetic approach that identifies disease genes by progressive dissection of linkage regions that are consistently co-inherited with the disease [19]. Bone morphogenetic receptor 2 (*BMPR2*) was a class example to be identified as a gene underlying familial IPAH by linkage analysis [20]. BMPR2 belongs to family of the transforming growth factor β (TGF-β) super family. Other family members of TGF-β can be studied as candidate approaches of pulmonary hypertension. Genome wide association study (GWAS) is a powerful approach to overcome the limitations of candidate gene and positional cloning studies for identifying the genetic causes of complex traits. It examines the relationships between disease status or associated traits and allele frequencies with a large number of genetic polymorphism markers across the genome. It is efficient to screen the entire genome simultaneously. GWAS provides the opportunity to identify novel mechanisms of disease pathogenesis that are caused by previously unsuspected genes or regulatory regions. More than 2,000 associations for more than 300 complex diseases and traits were identified in recent years by GWASs [21]. The strength of GWAS is that many novel genes that were never suspected to have roles in the diseases were identified.

Epigenetic effect is other possible cause of familial clustering. It regulates gene expression that is not caused by changes in the DNA sequence in the genome but by DNA methylation, histone modification and other mechanisms. The patterns of gene expression that determine cellular types and function are due to methylation of CpG sequences and gene silencing. Age, sex and other environmental factors have all been strongly associated with altered methylation in the genome. Histones are highly alkaline proteins in eukaryotic cell nuclei that package the DNA into nucleosome. Methylation, acetylation, phosphorylation, ubiquitination and sumoylation are major histone modification. The modifications can affect specific genes from activation to inactivation or from inactivation to activation in the specific cells or tissues.

Transcriptional profiling is to quantify the transcriptional level (RNA level) of the genome in cells or tissues. There are two methods for systematic quantification of RNA in cells or tissues; one is microarray systems and the other is RNA sequencing. The advantage for RNA sequencing is that it can possible identify the regulation non-coding RNA (ncRNA) in the relevant cells or tissues. Noncoding RNAs include housekeeping RNAs, long noncoding RNAs and small noncoding RNAs and emerged as novel molecules that are important in human complicated diseases in recent year. Micro RNAs (miRNAs) are the most studied small ncRNAs. miRNAs are about 18-25 nucleotide long noncoding RNAs that silence target mRNA for degradation and then inhibiting the translation. miRNAs can target 60% of mRNAs and control the signally pathways in most cell types [22].

Genetics and genomics progress of pulmonary hypertension

In 2000, linkage analysis for candidate genes identified *BMPR2* as a major gene underling familial IPAH [20,23]. The mutations of the gene are responsible for more than 70 percent of heritable pulmonary arterial hypertension (HPAH) cases and approximately 20 percent of IPAH cases [24,25]. It has more than 300 mutations in the locus which represent missense, nonsense, deletion, frame shift mutations [26,27]. *BMPR2* is located on human chromosome 2q33. The gene has thirteen exons and translates 1038 amino acids for the molecular weight of 115201 Daltons. *BMPR2* encodes a member of the bone morphogenetic protein (BMP) receptor family of transmembrane serine/threonine kinases. The ligands of this receptor are BMPs. These ligands and other accessory molecules, activins are also members of the TGF-β superfamily. BMPs are involved in endochondral bone formation and embryogenesis. These proteins transduce their signals through the formation of heteromeric complexes of two different types of serine (threonine) kinase receptors: type I and type II receptors. In a recent study 30 variants in the *BMPR2* gene were analysed for the function roles. Minigene assays identified that 6 variants (synonymous, missense) to result in splicing defect, 4 mutations to affect the protein localization and 4 mutations located in the 5'UTR region to show a decreased transcriptional activity in luciferase assays [28].

Except *BMPR2*, the mutations of other TGF-β family member *SMAD8* were also reported to have associations with PAH [29]. Direct sequencing identified four variants in *SMADs* 1, 4, and 9 among a cohort of 324 PAH cases [30]. The polymorphisms of another type I receptor *BMPR1* are associated childhood IPAH [31]. *KCNK3* is a gene codes potassium channel, exome sequencing identified it's mutations to have association with HPAH [32].

A genome-wide association study (GWAS) was conducted based on two independent case-control studies for idiopathic and familial PAH (without *BMPR2* mutations). A significant association at the cerebellin 2 precursor (*CBLN2)* locus on 18q22.3 was identified, with the risk allele conferring an odds ratio for PAH of 1.97. CBLN2 is expressed in the lung, and its expression is higher in explanted lungs from individuals with PAH and in endothelial cells cultured from explanted PAH lungs [33].

The mechanisms for development of pulmonary hypertension (PH) could involve increased pulmonary flow with the left-to-right shunts with congenital heart disease (CHD), upper airway obstruction, dysfunctional vascular smooth muscle cells with hyperproliferation leading to pulmonary vessel stenosis and remodelling [27]. For example, Gaucher disease has pulmonary hypertension and is reported to response well to the enzyme replacement therapy [34]. In microcephaly thyroid, sensorrineural abnormality, and other mental retendered syndrome that have PH, deletions of *TBX2* and *TBX4* at human chromosome 17q23.2 were identified [35,36]. Activin A receptor type II-like kinase-1 (*ACVRL1*, also known as *ALK1*) mutation is responsible for hereditary hemorrhagic telangiectasia (HHT) and heritable PAH. ALK-1 is a type I receptor for TGF-β signalling superfamily [37] and its polymorphisms were also associated with the PAH and play critical roles in the pathway to the integrity of the pulmonary vasculature [38,39].

Micro RNA and other epigenetic factors also have important roles in pulmonary arterial hypertension. It suggested normal *miR204* levels might present a novel therapeutic approach for human PAH [40]. *miRNA-17-5p* and *miR20a* targeted *BMPR2* and *miR-17/92* regulated *BMPR2* as well [41]. BMPR2 with significantly down-regulated expression in many PAH lungs, even in the absence of a germ-line mutation [42]. Epigenetic investigation in pulmonary hypertension found *SOP2* promoter was hyper-methylation in two CpG positions [43]. Histone deacetylases 1(HDAC1) was dramatically elevated in pulmonary arteries of human with pulmonary hypertension [27]. In PAH, plasma metabolomics approach showed modified transfer RNAs and altered bioenergetics [44].

Pulmonary venous hypertension (PVH) happens when the heart can't efficiently carry blood away from the lungs. This results in blood collecting in lung tissue. It is generally caused by diseases of the left side of the heart. Endomyocardial fibrosis (EMF) is such a condition and is a neglected tropical disease that affects an estimated 10 million people

worldwide [45]. In a genetic study, HLA alleles associated with cases of EMF in two populations from sub-Saharan Africa were identified, with EMF patients being more likely to have the HLA-B*58 allele in Mozambique and the HLA-A*02 in Uganda. Further investigations are needed to understand the role of genetics in EMF development [46].

Genetics and genomics progress of pulmonary embolism

Pulmonary embolism is also a complicated disease that many genes may contribute its cause [47,48]. The thrombophilic state is cause by an imbalance between the pro-coagulant and anticoagulant components of the coagulation system, resulting in an increased tendency to thrombosis [14]. The most common inherited or genetic risk factor for VTE is factor V Leiden (FVL) gene mutation on human chromosome 1q [49]. A point mutation in the factor V gene that causes a missense mutation in the protein (Arg506Gln, factor V Leiden) leads the molecule less susceptible to inactivation by activated protein C (APC) to cause thrombophilia and intravascular coagulation disorders [50]. The mutation is found in 3–7% of the healthy Caucasian population [51].

Prothrombin (factor II) is the precursor of thrombin, the end product of the coagulation cascade. It is a vitamin K-dependent protein which is synthesized in the liver. Vitamin K acts as a cofactor for posttranslational gamma-carboxylation of prothrombin which is required for functional activity. G20210A mutation in the prothrombin gene (*PTM*) was first discovered in 1996 [52]. The mutation causes an increase in mRNA production due to the transitions among guanine–adenine nucleotides in 20210 position, leading to increase prothrombin level and the risk of thromboembolic disease [53]. A mildly elevated homocysteine (Hcy) level is generally accepted as a risk factor as well for atherosclerosis and thrombotic tendency [54]. Hyperhomocysteinemia may result from inherited defects in the controlling enzymes of Hcy metabolism. Methylenetetrahydrofolate reductase (MTHFR) plays a role in the transmethylation of homocysteine to methionine. A common 677C to T mutation in the *MTHFR* gene was found to decrease the enzymatic activity. Homozygosity for the *MTHFR* C677T mutation has been associated with an increase in blood clotting together with plasma homocysteine increase and DVT occurrence risk [55]. Deficiency of protein C and protein S, both are antithrombin, are the other causes for thrombophilia [14]. In a recent report, variants in one of the three anti-coagulant genes: *SERPINC1* (Antithrombin III), *PROC*, and *PROS1* were responsible for the death of idiopathic fatal pulmonary embolism (IFPE) [56].

Normal platelet aggregation requires an intact fibrinogen receptor; this receptor consists of two glycoproteins, GPIIb and GPIIIa. The platelet GPIIB/IIIa receptors have important roles in thrombus formation [57]. The polymorphism of $PI^{A1/A2}$ of *GPIIIa* had shown association with pulmonary embolism [58]. Plasminogen activator 1(PAI-1) had high level in PE and it may be associated with the risk of thrombosis due to inhibition of fibrinolysis [59].

The future perspective of investigation of pulmonary vascular diseases

The genetic and genomic approaches to pulmonary vascular diseases brought fruitful results for the diseases diagnosis and management in clinic. For the genes that confer the diseases, it is important to understand the functional roles in the diseases. Cellular model, mouse model and translational studies are all necessary tools for identifying the novel genes function and the pathways they regulate in human tissue and cells. Co-immunoprecipitation with mass spectrum analysis is also likely to reveal the working partners with the new molecules. Proteomic approach includes liquid chromatography and tandem mass spectrometry (MS/MS) and can be used comprehensively for proteins and their biological function. The pharmacogenomics approach will apply to identify the polymorphisms of the novel genes in the search for novel drug. A knockout mouse is a genetically engineered mouse in which one or more genes have been made inoperative. A conditional knockout approach allows researchers to delete the gene of interest in a time- and space dependent manner. The development of CRISPER/Cas system offers an effective means for genomic editing [60].

Current epigenetic research and single cell sequencing can determine the contributions of genetic heterogeneity in pulmonary vascular diseases development or treatment response [61]. The precision medicine approaches for the diseases just began to emerge [62]. With the development of the genomic technology, it is reasonably optimised that in the near future, pulmonary vascular diseases will be effectively managed in clinic with good medical care for the prevention and treatment.

PAH remains a devastating disease for incident cases with significantly reduced survival [63]. PE is a relatively common cardiovascular emergency. It is important to understand the regulations of the pathways that determine the conditions. Currently there is no therapy to have demonstrated ability to reverse or cure PAH. There is also a profound need to further our pathophysiologic knowledge from genomics and genetics to promote novel therapeutic development [64].

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