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Genetic relationship between pulmonary diseases of environmental or occupational origin and osteoporosis: a bioinformatic approach

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Summary

Objetives: Identifying biomarkers that relate osteoporosis to occupational and environmental lung diseases.
Material and methods: Using integrated medical terminology databases, diseases related to lung diseases were obtained which, together with osteoporosis, were analyzed in DisGeNET to obtain the genes associated with each disease and form a protein-protein interaction network (PPI) through the Cytoscape StringApp. Applying different centrality algorithms using CythoHubba in Cytoscape, the 5 network proteins with the highest degree of centrality were selected.
Results: 9 diseases were included in the group of pulmonary diseases. 2,698 genes associated with lung diseases and osteoporosis were obtained. Genes associated with osteoporosis and with at least two of the included lung diseases resulted in a PPI network with 152 nodes and 1,378 axes. The proteins with the highest degree of network centrality were AKT1, ALB, IL6, TP53 and VEGFA.
Conclusions: There is a significant relationship between osteoporosis and the environmental lung diseases studied, through genes with dual involvement. We propose five important genes that link these diseases. This could provide a coherent basis for further research in this field.

Key words: osteoporosis, air quality, air pollution, lung disease, biomarkers, bioinformatics.

Introduction

Osteoporosis is currently defined as "a systemic skeletal disease characterized by decreased bone mass and a structural alteration of the bone tissue that determines a decrease in bone resistance resulting in a significant increase in fragility and susceptibility to fractures"[1]. The classic risk factors associated with the development of osteoporosis are age, a history of previous fracture or family history of osteoporosis, and prolonged estrogen deficiency[2].

On the other hand, one of the most important risk factors for mortality at the population level is air pollution. The consequences derived from air pollution have a high economic and social impact. In 2015, the costs derived from pollution-related morbidity and mortality reached 21 billion dollars worldwide and an estimated number of premature deaths between 6 and 9 million people in 2060 due to outdoor air pollution[3]. Air pollution has been shown to have a direct impact on health, causing various adverse effects[4]. The relationship between air pollution and environmental lung diseases has been reported in numerous studies[4-6]. Likewise, there is strong scientific evidence that relates poor air quality in different work environments with the development of different respiratory diseases[7,8].

In the 1980s, the first studies were published that showed an association between air quality and bone quality, observing a significantly higher incidence of fractures in city dwellers compared to those residing in rural areas[9-11]. Since then, studies in this line have increased notably over the last few years, indicating that prolonged exposure to air pollution is linked to a decrease in bone quality[12-14], and

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considered osteoporosis and fractures a modifiable risk factor[15]. However, a recently published systematic review points to the existence of inconsistent associations between air pollution and osteoporosis risk, which could be explained by the heterogeneity in the characteristics of the subjects participating in the related studies published[16].

Despite the fact that scientific evidence suggests the relationship between lung diseases and osteoporosis, currently no special attention is paid to air quality as a potential bone health problem. Considering the increase in air pollution in recent years, delving into this topic to understand the cross paths between the harmful effects of air pollution and bone health is urgent.

In this context, the identification of robust genetic markers that are present both in environmental and occupational lung diseases related to pollution and air quality, as well as in diseases related to bone quality, will allow the development of preventive and therapeutic strategies focused on the population at highest risk. For this type of study, bioinformatics plays an important role, allowing us to identify, through the use of different tools and algorithms, molecules that can act as common biomarkers between different diseases. In this sense, our study aimed to build and analyze a protein-protein interaction network relating genes involved in different environmental and occupational lung diseases related to pollution and air quality, with genes associated with the development of osteoporosis, to identify biomarkers common in both diseases.

Material and methods

Data collection

First, a literature search was performed to obtain a selection of unified terms to determine pulmonary and occupational diseases. The unified medical terminology databases consulted were Unified Medical Language System (UMLS, https://www.nlm.nih.gov/research/umls/index.html) y Medical Subject Headings (MeSH, https://www.nlm. nih.gov/mesh/meshhome.html). Subsequently, each of the selected diseases was used to determine disease-associated genes (GDA) by using the platform DisGeNET (http:// www.disgenet.org/home/), which integrates information on the relationship between genes and diseases from various sources of public data and literature on gene expression, biomarkers, association between variants and diseases, single nucleotide polymorphisms and associations of clinical phenotypes with the corresponding diseases[17].

Selection of seed genes

Once the genes associated with the different lung diseases (of environmental or occupational origin) and osteoporosis had been obtained, an initial filtering was carried out selecting those genes common to osteoporosis and with at least one of the lung diseases framed within this study. In a second phase, those genes associated with osteoporosis and with two lung diseases were selected to generate the protein interaction network (PPI).

Construction of PPI networks

To build the PPI network of genes associated with the diseases under study, the STRING application was used (1.6.0 version, released: 8 Sep 2020, http://apps. cytoscape.org/apps/stringapp)[18] within the Cytoscape platform (versión 3.8.2, https://cytoscape.org/)[19]. The confidence cut-off point value was established at 0.7. Only those interactions with a value greater than or equal to 0.7 were considered significant. Proteins below this cut-off point and those unrelated to other proteins were discarded.

Study of centrality and identification of genes

Obtaining the central genes, that is, those important nodes with a high number of interactions towards other nodes, were selected using the CytoHubba complement in Cytoscape[20]. Five calculation methods were used: Degree, Betweennes, Maximal Clique Centrality (MCC), Bottleneck and Closeness[21]. The common genes derived from these six algorithms represent key candidate genes with important biological regulatory functions.

Results

Data collection

After reviewing the unified medical terms, the diseases included in table 1 were selected. These were used to compare the genes associated with each of them with osteoporosis. Diseases in which genes associated with the disease had not been identified were not included.

Selection of seed genes

After applying the criteria established for the inclusion of genes in the PPI network, 157 genes associated with osteoporosis and at least two lung diseases were obtained. The complete list can be viewed in the supplementary material.

Construction of PPI networks

The generated PPI network included 152 proteins (nodes) and 1,378 relationships (axes). Of this set, 12 proteins were discarded because they were not linked to other proteins (figure 1).

Table 1. Unified terms for diseases of this study and unique identification code

*SLMU CUI	Unified Term/Pathology
C0003165	Anthracosis
C0003849	Asbestosis
C0006542	Byssinosis
C0024117	Chronic obstructive airway disease
C0025500	Mesothelioma
C0029456	Osteoporosis
C0037116	Silicosis
C0206062	Interstitial lung disease
C0264423	Occupational asthma
C0340092	Summer-type hypersensitivity pneumonitis

*SLMU: unified medical language system; CUI: unique identification code.

Study of centrality and identification of genes

10 central genes were determined by means of the CytoHubba application from the centrality methods detailed in table 2. Next, the 5 genes that appeared most frequently in the different centrality algorithms were selected (table 3), which shared their presence in all the algorithms used, evidencing their importance within the constructed interaction network.

Discussion

Any annual report on air quality reveals unflattering conclusions. The World Health Organization constantly warns about non-compliance with recommended standards for achieving healthy air quality. Poor air quality has a great health impact, causing 4.2 million premature deaths a year worldwide, mainly associated with exposure to small particles with a diameter less than or equal to 2.5 microns[22]. The main health effects of exposure to an environment with poor quality or polluted air are known and have been scientifically proven for many years. These include lung cancer, respiratory and cardiovascular diseases, stroke, etc. However, there are other less known effects or with little scientific evidence associated with the lack of studies in this area.

Due to the aging of the population, osteoporosis is considered one of the most prevalent diseases in the population worldwide. Currently, the association between poor air quality and osteoporosis is not well defined in the scientific literature, but it is beginning to be of great importance due to the health impact of the high percentage of the population with osteoporotic problems or low bone quality.

The results of this study show a set of genes associated with the presence of both lung diseases (derived from environmental contamination or associated with certain occupational activities) and osteoporosis. Pulmonary diseases are mainly caused by repeated exposure over time to chemical irritants, allergens or toxins, which can cause lasting effects on the individual.

The bibliographic search of pulmonary diseases of environmental or occupational origin, offered a set of diseases that, without being a priori related to osteoporosis, could share one or more genes linked to osteoporotic processes. Thus, we find anthracosis, a lung condition produced by exposure and inhalation of dust with a high concentration of carbon; asbestosis, in which asbestos is the main inhaled particle; byssinosis, produced by inhaling cotton dust among other particles of plant origin; chronic obstructive pulmonary disease (COPD), a consequence of long-term exposure to irritants such as polluted air, chemical fumes or dust, among others; mesothelioma, a specific form of lung mesothelial cancer that is usually related primarily to occupational exposure to asbestos; silicosis, caused by inhaling dust with silica particles; interstitial lung diseases, which encompass a group of lung diseases most of which are caused by progressive scarring of the lung tissue due to prolonged exposure to dangerous substances (such as certain organic substances, wood, metals, infectious agents such as viruses, drugs, etc.); occupational asthma, produced by inflammation of the pulmonary airways caused by inhalation of substances produced in the workplace such as wood dust, fungi, and/or chemical substances, among others, and hypersensitivity pneumonitis, caused by exposure to a large number of organic particles such as fungi or bacteria, causing a significant inflammatory response[23].

This set of diseases derived from poor air quality has been related to osteoporosis, reflecting a high genetic relationship between the two types of diseases. The results of the present study are consistent with previous studies on the association of respiratory diseases with osteoporosis[12-14]. In addition, especially relevant genes have been obtained that could be considered as potential common markers between pulmonary and osteoporotic diseases.

Figure 1. Protein-protein interaction network of genes associated with the lung diseases of this study and linked to osteoporosis

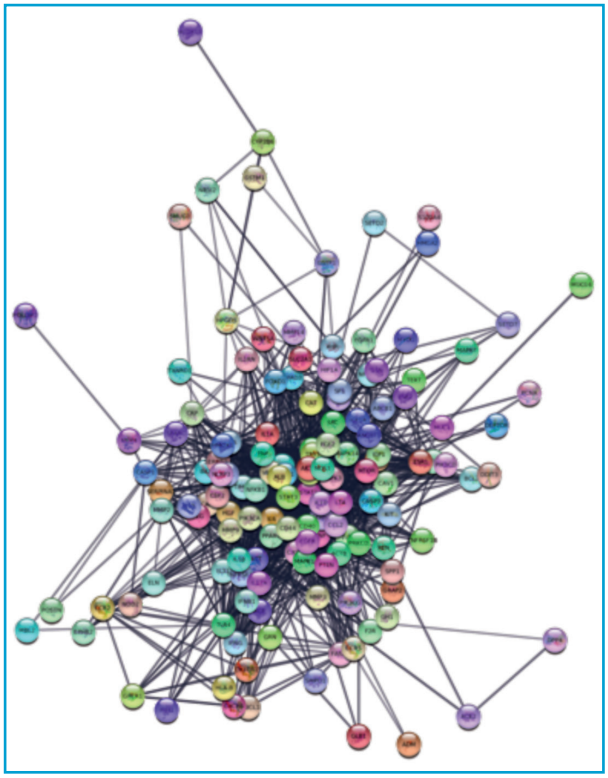

Table 2. Ranking of genes of the biological network according to the centrality algorithm used

Degree	Betweenness	*MCC	Bottleneck	Closeness
TNF	TNF	TNF	VEGFA	TNF
VEGFA	SRC	VEGFA	AKT1	VEGFA
AKT1	VEGFA	AKT1	HGF	AKT1
MAPK3	AKT1	MAPK3	SPP1	MAPK3
IL6	MAPK3	IL6	IL6	IL6
EGFR	IL6	MAPK8	TP53	MAPK8
TP53	EGFR	TP53	VCAM1	EGFR
CXCL8	TP53	CXCL8	CAT	TP53
ALB	ALB	CCL2	ALB	ALB
STAT3	MAPK1	ALB	MAPK1	STAT3

*MCC: maximal clique centrality.

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Table 3. Ranking of Genes with more presence in the applied centrality algorithms

Genes	Unified name
AKT1	AKT Serine/Threonine Kinase 1
ALB	Albumin
IL6	Interleukin 6
TP53	P53 tumor protein
VEGFA	Vascular endothelial growth factor A

In systems biology, discovery of major proteins and their corresponding molecular pathways in complex diseases is booming thanks to PPI network analysis and enrichment analysis. The systems biology approach to investigating disease-associated biology is revolutionizing the understanding of cellular pathways and gene networks that underlie the onset of diseases, thus facilitating the discovery and development of new drugs and therapies thanks to the identification of markers in disease progression.

In biomolecular networks, where genes and/or proteins are nodes and the molecular interactions are the edges that interconnect the nodes, the importance of a node can be measured by the effect produced in the changes in the function of the interaction network. after deleting that node. These essential nodes are called hubs. In this study, 5 hubs have been determined that could consistently disturb the interaction network and that could cause important effects on the body's fitness. The hubs that have been selected and that relate pulmonary diseases of environmental or occupational origin with osteoporosis are AKT1, ALB, IL6, TP53 and VEGFA, table 4. However, future studies could suggest new hubs that present a lower score in the classification performed in this study.

The role that these hubs play in osteoporotic processes and lung diseases is well known and corroborates the importance of the genes selected in this work to relate the diseases under study.

In the case of the AKT1 gene, which encodes the protein called AKT1 kinase, it is found in various types of cells, playing important roles in many signaling pathways; in the case of osteoporosis, AKT1 acts as a negative regulator of osteoblast differentiation and as a positive mediator of osteoclastogenesis. It is therefore considered a potential target as a therapeutic target to improve osteoblast differentiation and bone mass formation while limiting osteoclast development and bone resorption[24,25]. This protein also has pulmonary involvement, participating in the appearance and progression of pulmonary fibrosis, by promoting the differentiation of myofibroblasts and the deposition of proteins in the extracellular space and on the other hand, through the regulation of TSP1. Due to this, AKT1 has been proposed as a biomarker of interstitial lung disease[26], so that the use of AKT1 inhibitors such as triciribine, is considered as a potential therapeutic strategy for the treatment of these diseases. These data corroborate its dual role in both conditions.

Furthermore, the ALB gene encoding albumin was identified. Low levels of this protein have been associated with an increased risk of fractures due to low bone mineral density (BMD), with a positive correlation between serum albumin and BMD[27,28]. In relation to lung diseases, an association between albumin and interstitial lung disease has been shown, with significantly lower levels of albumin being observed in patients affected with interstitial lung disease[29].

Interleukin 6, encoded by the IL6 gene, is considered a key factor in postmenopausal osteoporosis due to its ability to activate osteoclasts and induce bone resorption[30]. Furthermore, it has been identified as a promising target for osteoporotic treatment due to its fundamental role as an inhibitor of osteogenesis and as a predictor of bone loss[31,32]. In relation to lung diseases, we can highlight studies that correlate the overexpression of IL6 with COPD[33,34], although there are others that point in the opposite direction[35]. With regard to silicosis, there are several studies that show increased levels of this cytokine from early stages of the disease, being considered as a potential biomarker for the early diagnosis and treatment of patients with silicosis[36]. Likewise, there are studies that relate the increase in IL6 levels with the development of anthracosis. In this sense, several studies show an association of certain IL6 polymorphisms with the development of anthracosis and/or anthrosilicosis as well as with associated genotoxic effects[37,38].

With regard to the tumor protein p53, encoded by the TP53 gene, there are several studies that relate it both to osteoporosis and to different lung diseases. In this context, several studies show that increased serum levels of p53 are associated with a decrease in bone mass, and the suppression of p53 partially reverses the decrease in BMD in vitro and in vivo[39]. In the case of lung diseases, it plays a leading role in the development of COPD, with increased levels of this protein being observed in affected patients[40]. Likewise, an association between p53 and mesothelioma has been observed[41]. In this sense, it has been reported that approximately 15% of patients affected with mesothelioma present some type of mutation in the TP53 gene. In the same way, in approximately 25% of human mesotheliomas there are co-deletions of the TP53 genes together with PTEN and/or CDKN2A/p14ARF, associating the cooperative losses of these genes with the development of a significant proportion of these aggressive neoplasms[42]. These findings may lead to the establishment of appropriate therapeutic strategies for the joint treatment of both types of diseases.

Lastly, the VEGFA gene encoding vascular endothelial growth factor A is known to play an important role in bone biology by participating in endochondral ossification. Furthermore, VEGFA is expressed at high levels in osteoblast precursors and can stimulate osteogenic differentiation in various types of cells[43]. On the other hand, decreased levels of VEGFA are reportedly associated with osteoporosis through an intracellular mechanism possibly mediated by the regulation of the transcription factor RUNX2, considered as a therapeutic target of the disease[44]. This gene has also shown relevant activity in lung diseases, being an autocrine growth factor in mesothelioma and a potent mitogen for mesothelial cells[45].

VEGFA is involved in angiogenesis and stimulates neovascularization of tumors. In malignant mesothelioma, elevated levels of VEGFA and its receptor have been detected by immunohistochemistry[46,47] and have been correlated with microvessel density, increased tumor necrosis, and worse survival[48]. In this sense, drugs aimed at blocking VEGFA, such as bevacizumab, have recently shown their efficacy in the treatment of mesothelioma[49].

Despite the amount of information on the role of these genes in certain pulmonary and osteoporotic diseases, there are few studies that relate both types of diseases, therefore, it is necessary to delve into these aspects in order to identify possible common therapeutic targets. The association between lung diseases (triggered by air pollution or poor air quality in work environments) and bone quality could open the door to the design of behavioral strategies aimed at modifying the lifestyle that implies an improvement in bone health. On the other hand, it is important to consider the population with some type of lung disease, as a population at risk of bone fragility. Therefore, the knowledge provided by this type of study reveals the importance of studying BMD in people with lung diseases to establish early therapeutic and preventive measures in order to reduce the risk of fractures in this vulnerable population.

Table 4. Genes with a high score in centrality associated with the pulmonary pathologies used in this study

Genes	Unified name
AKT1	[a]COPD, Mesothelioma, Silicosis, [b]PID
ALB	COPD, PID
IL6	COPD, Mesothelioma, Silicosis, PID, Anthracosis
TP53	COPD, Mesothelioma, Silicosis, PID
VEGFA	COPD, Mesothelioma, PID

[a]COPD: chronic obstructive pulmonary disease; [b]PID: pulmonar interstitial disease.

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