GENETIC POLYMORPHISMS ON THE RISK OF ASBESTOSIS: A SYSTEMATIC REVIEW

POLIMORFISMOS GENÉTICOS NO RISCO DE ASBESTOSE: UMA REVISÃO SISTEMÁTICA

MARCOS CESAR SANTOS DE **CASTRO**[1,2,*], KAIO CEZAR RODRIGUES **SALUM**[1,3], JÔNATAS DE MENDONÇA **ROLANDO**[1,3], CÍCERO BRASILEIRO MELLO **NETO**[1], ÂNGELA SANTOS FERREIRA **NANI**[2], FABIANA BARZOTTO **KOHLRAUSCH**[3]

1. Post-Graduate Program in Science and Biotechnology, PBBI, Fluminense Federal University, Niterói, RJ, Brazil; **2.** Department of Clinical Medicine, Antônio Pedro University Hospital, Fluminense Federal University, Niterói, RJ, Brazil; **3.** Laboratory of Human Genetics, Fluminense Federal University, Niterói, RJ, Brazil.

* Hospital Universitário Antônio Pedro. Avenida Marques do Paraná, 303 - Centro, Niterói, Rio de Janeiro, Brazil. Zip Code: 24033-900. marcos_cesar@id.uff.br

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ABSTRACT

Background: Asbestosis is an irreversible pulmonary fibrosis with long latency time and variable clinical manifestations. Genetic variants might affect the functions of proteins involved in the pathophysiology of the disease. Methods: We performed a systematic review using PRISMA statement criteria to identify association studies of genetic polymorphisms and asbestosis, published in English, from 1987 to December 2022. Data sources included PubMed, Scopus and ISI Web of Science databases. Results: Our preliminary search identified a total of 377 articles and after the assessment of the inclusion and exclusion requirements, ten articles were selected. These studies observed statistically significant associations between polymorphisms in *TNFα, GSTP1, GSTT1, SOD2, A1AT* genes and asbestosis. Interactions between polymorphisms in *CAT* and *SOD2,* and *CAT* and *iNOS* also demonstrated association with asbestosis. Conclusions: Due to the scarcity of studies on the subject and the lack of replication of the results of these studies in different populations, these associations should be carefully evaluated, and further studies are needed to verify these findings.

KEYWORDS: association study, genetics, polymorphism, susceptibility, asbestosis.

1. INTRODUCTION

Pneumoconiosis are lung diseases resulting from inhalation and deposition of dust or mineral particles in the organ, leading to tissue reaction and fibrosis of the lung parenchyma[1]. The development and severity of pneumoconiosis depend on several factors, but the most relevant are the amount of inhaled dust or particles, which will directly depend on the concentration, exposure time, size, shape and physical-chemical characteristics of the particles, the integrity of lung defenses, and individual factors, including genetic characteristics[2,3].

Asbestos-related diseases (ARD), caused by asbestos (amianthus) fibers inhalation, are among the most prevalent pneumoconiosis. ARD are characterized by pleural and/or pulmonary parenchymal involvement as pleural effusion, circumscribed pleural thickening or pleural plaques, diffuse pleural thickening, round atelectasis, lung cancer, malignant pleura mesothelioma and asbestosis[1].

There is no safe concentration level for asbestos exposure[4], and although it has been banned in 55 countries, it is still widely used worldwide[5]. Approximately 2 to 6 million people are exposed to asbestos fibers in United States[6], causing 12 to 15 thousand deaths per year[4]. Although amianthus extraction has reduced substantially in developed countries in recent years, it is important to highlight environmental and non-occupational exposure, which is increasing in activities such as “do-it-yourself”, in which many activities are done without adequate protection, such as home cutting and drilling materials containing asbestos[7]. The effects of the implementation of measures to ban asbestos can only be observed in approximately 20 years, due to the long latency time for the onset of the disease[8].

Among the ARD, asbestosis deserves special attention due to the possibility of severe clinical, radiological, and functional impairment. Asbestosis is defined as an irreversible fibrosis of the pulmonary parenchyma, characterized by a bilateral reticular interstitial infiltrate caused by asbestos fibers inhalation[5,6]. The development of asbestosis shows a significant dose-response relationship to exposure. Individuals with low exposure to asbestos often develop mild illness, with relative stability over the years. Patients exposed to high concentrations of asbestos develop a more extensively pulmonary parenchyma involvement with more intense progression[7].

The mechanism of lung injury caused by asbestos fibers is not fully established[9,10]. When present in the alveolar space, fibers induce the production of reactive oxygen species (ROS), which can lead to DNA damage, and, if not effectively repaired, promotes cell apoptosis, gene mutations and chromosomal changes[10].

Differences in individual's genetic susceptibility could modify on the susceptibility, onset, and severity of asbestosis[7,11]. The investigation of genetic polymorphisms to identify markers to asbestosis susceptibility has been conducted in the last decades. Therefore, this study aims to conduct a systematic review on studies evaluating the effect of genetic polymorphisms on asbestosis.

2. MATERIALS AND METHODS

A systematic review process was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement criteria (PRISMA) [12]. The main scientific databases (PubMed, Scopus and ISI Web of Science) were used to identify association studies between genetic polymorphisms and asbestosis, published in English, from 1987 to December 2022. The search was performed using the descriptors "genetic polymorphism" combined with "asbestosis", "amianthus", "pneumoconiosis", or "occupational diseases". The descriptors were combined with the "AND" operator. All titles and abstracts retrieved by computerized research were independently reviewed by two authors (Castro and Kohlrausch), who selected the articles relevant to the objectives of the review, according to the inclusion criteria.

The inclusion criteria were: (a) association studies of genetic polymorphisms and asbestosis, (b) case-control studies, (c) studies developed with humans exposed to asbestos fibers, (d) studies in which genes and polymorphisms were clearly described, with results expressed in terms of odds ratio (OR) and 95% confidence interval (CI), and (e) studies in which occupational exposure to amianthus was confirmed by occupational employment histories in any industry sector, collected through medical records, self-reported information by patients and environmental monitoring. No limits were adopted for the duration of occupational exposure to amianthus, and no restrictions were imposed on the geographic areas of investigation, patient origin or statistical methods used by investigators.

The exclusion criteria were: (a) reviews, (b) case reports, (c) conference articles, letters, commentaries, or editorial materials, (d) experimental studies on cell and animal models, (e) publications not focusing on asbestosis exclusively, (f) epidemiological studies not related to the issue, and (g) publications in languages other than English, even if the abstract was in English.

All full texts of the articles considered adequate for the review were obtained and submitted to a critical evaluation. Each author (Castro and Kohlrausch) performed independent data extraction and all possible differences were solved by mutual consent.

3. RESULTADOS

The preliminary research identified 377 articles: 276, 65 and 36 in PubMed, Scopus, and Isi Web of Science databases, respectively. Seventy-four duplicates were removed from the total number of articles. Of the remaining 303 articles, the two authors excluded 246 articles independently, as they did not meet the inclusion criteria based on analysis of title and abstract. The remaining 57 articles were carefully evaluated in relation to their full content. After the evaluation, the two authors excluded 47 articles independently. At the end of the process, 10 articles were included in this review (Figure 1).

All included case-control studies were performed in Caucasian subjects from Slovenia (exposed patients with asbestosis n = 262; control group exposed to asbestos n = 265), Germany (exposed patients with asbestosis n = 395; control group with no history of exposure n = 177) and Spain (exposed patients with asbestosis n = 100; control group exposed to asbestos n = 94), and restricted to only three research groups (Table 1). These publications were concentrated between the years of 2007 and 2014. Classical genotyping methods were used in these studies, including polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Until now, there is no systematic review on the subject in the literature.

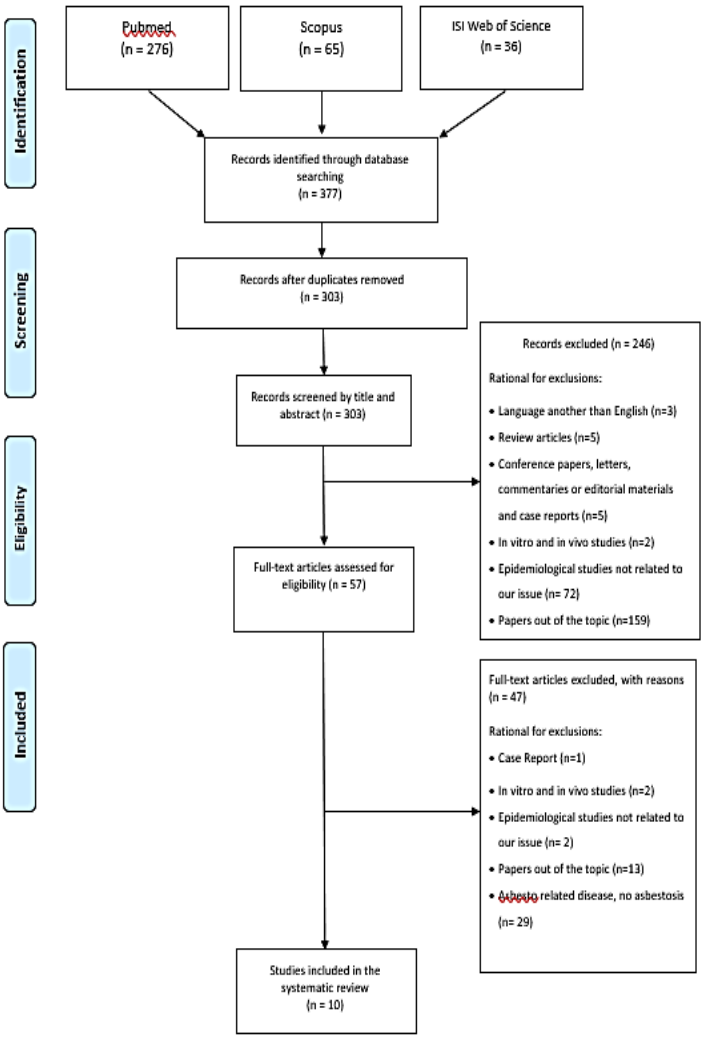

Figure 1. Flowchart of literature search and selection process (PRISMA).

Table 1: Summary results of nine association studies with gene polymorphisms and asbestosis.

Gene[a]	Allele/Genotype	Effect	Country	Sample size	*P*-value	Odds Ratio (95% CI)	Author
TNFα							
-238G/A	GA+AA	No effect	Germany	395 cases/177 controls[b]	0.59	1.18 (0.63-2.21)	Helmig et al.[20]
-308G/A	GA+AA	Susceptibility	Germany	395 cases/177 controls[b]	0.03	1.57 (1.03-2.36)	Helmig et al.[20]
TGFβ1							
Leu10Pro	Leu/Pro+Pro/Pro	No effect	Germany	401 cases/83 controls[b]	0.303	1.29 (0.79-2.11)	Helmig et al.[26]
Arg25Pro	Arg/Pro+Pro/Pro	No effect	Germany	401 cases/83 controls[b]	0.386	1.39 (0.68-2.83)	Helmig et al.[26]
iNOS							
$(CCTTT)_n$	LL	No effect	Slovenia	262 cases/265 controls	N.A.	1.20 (0.85-1.69)	Franko et al.[34]
SOD2							
Ala-9Val	Ala/Ala	Susceptibility	Slovenia	262 cases/265 controls	0.046	1.50 (1.01-2.24)	Franko et al.[16]
SOD3							
Arg213Gly	Arg/Gly	No effect	Slovenia	262 cases/265 controls	0.317	1.63 (0.62-4.27)	Franko et al. [16]
GCLC							
-129C>T	CC+TT	No effect	Slovenia	147 cases/178 controls	0.874	0.95 (0.57-1.67)	Franko et al.[41]
GCLM							
-590C>T	CT+TT	No effect	Slovenia	147 cases/178 controls	0.369	0.95 (0.63-1.43)	Franko et al.[41]
GSTP1							
Ile105Val	Ile/Ile	susceptibility	Slovenia	262 cases/265 controls	0.017	1.52 (1.08-2.15)	Franko et al.[43]
Ala114Val	Ala/Ala	No effect	Slovenia	262 cases/265 controls	0.897	0.97 (0.64-1.48)	Franko et al.[43]
Ile105Val +Ala114Val	A/A	susceptibility	Slovenia	262 cases/265 controls	0.023	1.49 (1.06-2.10)	Franko et al.[43]
Ile105Val	AG+GG	No effect	Slovenia	147 cases/178 controls	0.157	0.73 (0.47-1.13)	Franko et al. [41]
Ala114Val	CT + TT	No effect	Slovenia	147 cases/178 controls	0.482	0.82 (0.47-1.43)	Franko et al.[41]
GSTT1							
GSTT1-null	Null genotype	Protective	Slovenia	262 cases/265 controls	0.02	0.61 (0.40-0.94)	Franko et al.[44]
GSTT1-null	Null genotype	Protective	Slovenia	147 cases/178 controls	0.028	0.51 (0.28-0.93)	Franko et al.[41]
GSTM1							
GSTM1-null	Null genotype	No effect	Slovenia	262 cases/265 controls	0.96	1.01 (0.71-1.43)	Franko et al.[44]
GSTM1-null	Null genotype	No effect	Slovenia	147 cases/178 controls	0.721	1.13 (0.76-1.69)	Franko et al.[41]
A1AT							
Glu342Lis	Pi*Z	Susceptibility	Spain	100 cases/94 controls	0.04	8.9 (1.02-76.4)	Lafuente et al.[50]
Glu264Val	Pi*S	No effect	Spain	100 cases/94 controls	0.1	1.5 (0.80-3.05)	Lafuente et al.[50]
CAT							
-262C/T	TT	No effect	Slovenia	262 cases/265 controls	0.364	1.36 (0.70-2.62)	Franko et al.[15]
***CAT* interaction**							
CAT -262TT and *SOD2* Ala-9Val	*CAT* TT and *SOD2* -9Ala/Ala	Susceptibility	Slovenia	262 cases/265 controls	0.004	2.67 (0.57-13.07)	Franko et al.[57]
CAT -262TT and *iNOS* $(CCTTT)_n$	*CAT* TT and *iNOS* LL	Susceptibility	Slovenia	262 cases/265 controls	<0.0001	5.14 (1.30-20.36)	Franko et al.[57]

[a]See text for abreviations; [b]non-exposed healthy controls. N/A: not available.

4. DISCUSSION

The exposure to asbestos stimulates the production of ROS and Reactive Nitrogen Species (RNS), probably contributing to DNA damage and subsequent pulmonary toxicity [10]. The most important ROS involved in the pathogenesis of asbestosis are superoxide (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2). Asbestos fibers can stimulate the production of ROS by the participation of redox-active iron (Fe^{2+}, Fe^{3+}) present in asbestos that catalyzes the formation of OH^-, and by alveolar macrophages during the phagocytosis of asbestos fibers[13,14].

Oxidative stress can cause damage to cellular macromolecules; however, antioxidant defense enzymes that neutralize ROS are normally present in cells and act as protective factors to the oxidative process [15]. Superoxide dismutase, catalase and glutathione peroxidase constitute the primary antioxidant defense against oxidative stress[16,17]. The ROS produced by the presence of asbestos is also an important signal for the induction of transcription and production of inflammatory and fibrotic cytokines[16,17].

Hereafter, the genes studied in the articles selected in this systematic review will be discussed. The association studies were mainly based in the molecular mechanisms related to higher concentrations of ROS and cytokines[18,19] in the lungs, and they will be discussed by the mechanisms involved in the development of asbestosis: cytokines (TNFα and TGFβ1), oxidizing agents (NOS) and antioxidant agents (SOD2, SOD3, GSTP1, GSTT1, GSTM1, A1AT, and catalase).

Cytokines

Several inflammatory and fibrotic cytokines produced by alveolar macrophages during phagocytosis of asbestos fibers, stimulate the production of ROS [18]. Among these cytokines, the tumor necrosis factor alpha (TNFα) and the transforming growth factor beta 1 (TGFβ1)[18,19] were investigated in asbestosis.

Tumor necrosis factor

TNFα is a multifunctional proinflammatory cytokine produced primarily by monocytes, macrophages, and lymphocytes. It is related to several inflammatory effects, such as the activation of neutrophils and mononuclear cells, inducing the expression of adhesion molecules, cytokines and chemokines, and the regulation of a wide spectrum of biological processes, as cell proliferation, differentiation, and apoptosis[20]. Moreover, TNFα plays an important role in particle-induced inflammation, promoting the induction of adhesion molecules and the stimulation of other proinflammatory molecules, in addition to acting as an initiator of inflammatory processes in the lung, playing an important role in the development of fibrous interstitial lung diseases [21,22].

Variations in the promoter region of the *TNFα* gene have been associated with susceptibility to several lung diseases, including pneumoconiosis[20]. Two of these polymorphisms, -238G/A (rs361525) and -308G/A (rs1800629), are associated with increased expression of TNFα, in the presence of the variant allele[20,23].

Helmig *et al*. (2010)[20] analyzed the influence of these two polymorphisms in the risk of asbestosis in individuals from Germany. The authors observed that genotypes containing at least one variant (A) allele of the -308G/A polymorphism in *TNFα* were significantly associated with asbestosis (OR=1.57; 95% CI 1.03-2.36; P=0.03). In a subgroup of severe asbestosis patients with a greater extent of pulmonary fibrosis, the odds ratio was even higher (OR=4.15; 95% CI 1.06-16.16; P=0.04). However, after controlling by smoking (pack-years), age and sex, this significant association was lost. In addition, no significant association was observed between the -238G/A polymorphism and asbestosis (OR= 1.18; 95% CI 0.63-2.21; P=0.59) (Table 1).

Transforming growth factor β1

The human bronchial cells have a high concentration of TGFβ protein, but it is also expressed in alveolar macrophages, mesenchymal cells, airway, and vascular smooth muscle cells[24]. Asbestos fibers lead to an induction of TGFβ1[25,26], promoting the pathogenesis of pulmonary fibrosis, suppressing the immune system and inducing extracellular matrix components, resulting in excessive deposition of scar tissue and fibrosis[24,26].

The *TGFβ1* gene is located at 19q13.2, and two functional polymorphisms (+869T/C and +915G/C), leading to amino acid substitution (Leu10Pro and Arg25Pro, respectively), and the alteration of TGFβ1 production[27,28], were studied in asbestosis. Helmig *et al*. (2009)[26] evaluated the association of these polymorphisms with asbestosis in Germany individuals, and no significant associations were observed with genotypes of both polymorphisms (+869T/C: OR=1.29; 95% CI 0.79-2.11; P=0.303; +915G/C: OR=1.39; 95% CI 0.68-2.83; P=0.386). However, the authors observed a significant association between the presence of proline allele (Leu/Pro + Pro/Pro) of the polymorphism +869T/C in patients with asbestosis when compared to patients with asbestos-related lung cancer, after adjustment for smoke (OR=3.715; 95% CI 1.559-8.854; P=0.003)[26] (Table 1).

Oxidizing Agents

Nitric oxide (NO) is the most important factor in the pathogenesis of asbestosis due to its toxicity and participation in the oxidative environment. Because it is a free radical, NO reacts quickly with reactive oxygen species, promoting the formation of toxic metabolites, such as peroxynitrite. Peroxynitrite oxidizes thiols much more than H_2O_2 and initiates iron-independent lipid peroxidation. In addition, peroxynitrite plays an important role in cell signaling, cell proliferation and cell death [18].

Nitric oxide synthase

NO is synthesized by nitric oxide synthetase (NOS). Three isoforms of the nitric oxide synthase enzyme have been identified: neural NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3), with iNOS being frequently present in cases of chronic inflammation[29].

Studies indicate that asbestos fibers could cause upregulation of NOS activity and in the production of NO by alveolar macrophages and pulmonary epithelial cells[30,31]. Quinlan *et al.* (1998)[32] observed a temporal relationship between the production of NO by alveolar macrophages and pulmonary infiltration by neutrophils after inhaling asbestos fibers[32].

The human *iNOS* gene has several polymorphisms in its promoter region, including the variable number tandem repeat (VNTR) polymorphism of a CCTTT pentanucleotide ($(CCTTT)_n$). Functional in vitro and gene expression studies showed an increase in iNOS expression correlated with an increase in the number of CCTTT repeats, due to increased gene promoter activity[33].

Franko *et al.* (2011)[34] investigated the association of $(CCTTT)_n$ polymorphism and the risk of asbestosis in individuals exposed to asbestos fibers in Slovenia. Alleles of 6 to 18 repeats were observed in the total sample, with the different alleles classified as short (S; ≤11 repeats) and long (L; >12 repeats). Although some studies suggest an association between inflammatory and malignant diseases with the number of CCTTT repeats [35,36], the authors did not observe a significant association between this polymorphism and asbestosis (LL versus SL+SS; OR=1.20; 95% CI 0.85-1.69; P = not available) (Table 1).

Antioxidant Agents

Superoxide dismutases (SOD) together with catalase and glutathione transferases (GST) are the primary defense against this oxidative stress[16,17]. Another important antioxidant agent, alpha-1-antitrypsin, is an antiprotease that inhibits elastase, a powerful destructive mediator used by alveolar macrophages and neutrophils as a proteolytic enzyme[37].

Manganese and extracellular superoxide dismutase

The superoxide dismutase (SOD) catalyzes the conversion of the O_2^- into H_2O_2 and oxygen (O_2)[16]. The Manganese-SOD (MnSOD or SOD2) is an intramitochondrial enzyme containing manganese, while extracellular SOD (ECSOD or SOD3) contains copper (Cu) and zinc (Zn) and is predominantly found in extracellular spaces binding to components of the pulmonary cellular matrix. Literature suggests that SOD2 could promote a double role in relation to ROS levels. Although SOD2 is considered an antioxidative enzyme by removing the superoxide anion, paradoxically, the H_2O_2 generated by SOD2 is toxic because it can be converted to OH, if not removed quickly and effectively by the enzymes glutathione peroxidase and catalase. Thus, high levels of SOD2 activity could result in increased production of the radicals H_2O_2 and OH, which can cause cell damage[16].

In humans, SOD2 is encoded by the *SOD2* gene, located at 6p25[17]. The most common functional polymorphism in SOD2 occurs by replacing cytosine (C) to thymine (T) (201C/T, rs4880), resulting in the replacement of alanine to valine at the -9 position of the mitochondrial segmentation sequence (Ala-9Val). The -9Ala allele was associated with higher SOD2 expression and activity, which would increase the production of H_2O_2 and contribute to the accumulation of ROS[38,39].

The SOD3 is encoded by the *SOD3* gene, located at 4p15.2, and one polymorphism (896C/G, rs1799895) leads to the exchange of the amino acid arginine for glycine at position 213 of the protein (Arg213Gli) [17]. This polymorphism was associated with an 8-to-15-fold increase in the concentration of SOD3 plasma levels[40].

Franko *et al.* (2009)[16] investigated the influence of the *SOD2* Ala-9Val and *SOD3* Arg213Gli on asbestosis in workers exposed to asbestos in Slovenia and observed the frequency of the *SOD2* -9Ala/Ala genotype significantly higher in asbestosis patients than in controls, when compared to the other combined genotypes (Ala/Val and Val/Val) (OR=1.50; 95% CI 1.01-2.24; P=0.046). No significant association was observed for the Arg213Gli polymorphism in *SOD3* (OR=1.63; 95% CI 0.62-4.27; P=0.317). Therefore, the authors concluded that the -9Ala/Ala genotype in *SOD2* could increase the risk of developing asbestosis in workers exposed to asbestos (Table 1).

Glutathione S-transferases

Glutathione (GSH) is an abundant cellular antioxidant which has a major role in the protection against oxidative injury in cells and serves as a substrate of many antioxidative enzymes [41]. The antioxidant capacity of the glutathione system depends on enzymes involved in its biosynthesis, such as glutamate cysteine ligase (GCL), as well as of the detoxification enzymes, such as glutathione S-transferases (GSTs)[41].

Glutathione S-transferases (GSTs) are phase II detoxifying enzymes known to inactivate the production of ROS and reactive nitrogen species (RNS), catalyzing the conjugation of electrophilic components, and thus leading to a reduction in glutathione. The GST family comprises at least seven classes of GST cytosolic isoenzymes: alpha, mu, pi, sigma, theta, kappa and zeta[42].

The Glutathione S-transferase pi 1 (GSTP1) is the most prevalent GST in the human lung. The gene encoding this enzyme (*GSTP1*) is located at 11q13.2 and shows polymorphisms that result in a reduction in the enzyme's conjugation activity. One of these polymorphisms is characterized by the substitution of

adenine (A) for guanine (G) in nucleotide 313 in exon 5 (313A/G, rs1695), leading to the replacement of isoleucine (Ile) by valine (Val) in position 105 of *GSTP1* (Ile105Val). Another polymorphism is characterized by the replacement of cytosine (C) by thymine (T) in nucleotide 341 in exon 6 (341C/T, rs1138272), leading to the replacement of alanine (Ala) by valine (Val) at position 114 of the gene (Ala114Val)[43].

Franko *et al.* (2008)[43] conducted an association study in the asbestos exposed individuals from Slovenia. They observed that the frequency of the 105Ile/Ile genotype when compared to the frequency of the other combined genotypes (105Ile/Val + 105Val/Val) was significantly higher in the asbestosis group than in control group (OR=1.52; 95% CI 1.08-2.15; P=0.017). No significant associations were observed with the Ala114Val polymorphism (OR=0.97; 95% CI 0.64-1.48; P=0.897)[43]. In 2021, Franko *et al.* (2021)[41] analyzed the association of the same *GSTP1* polymorphisms (Ile105Val and Ala114Val) in a smaller sample of 147 patients with asbestosis and 178 exposed healthy controls from Slovenia and no significant associations were observed (OR=0.73; 95% CI 0.47-1.13; P=0.157, and OR=0.82; 95% CI 0.47-1.43; P=0.482, respectively (Table 1).

Additionally, based on the presence of polymorphisms in both codons (105 and 114), four *GSTP1* alleles (A, B, C and D) were also determined, resulting in seven different genotypes (A/A, A/B, A/C, A/D, B/B, B/C, C/C). As both B and C alleles are reported in the literature as having low catalytic activity when compared to A allele (high catalytic activity), the *GSTP1* genotypes were grouped according to the conjugation capacity of the enzyme: high (A/A), intermediate (A/B, A/C and A/D) and low (B/B, B/C and C/C). The frequency of the *GSTP1* genotype encoding the enzyme with high conjugation capacity (A/A) against the combined frequency of all the remaining genotypes was significantly higher in the asbestosis group when compared to control group (OR=1.49; 95% CI 1.06-2.10; P=0.023)[43]. Therefore, the authors concluded that GSTP1 with high conjugation capacity, encoded by the A/A genotype, was associated with an increased risk of asbestosis[43].

Glutathione S-transferase mu 1 (GSTM1) and theta 1 (GSTT1) are encoded by the genes *GSTM1* and *GSTT1*, located at 1p13.3 and 22q11.23, respectively. The most common polymorphism in the *GSTM1* and *GSTT1* genes occurs by the deletion of these genes and the null genotypes (homozygous for the deletion) result in the absence of protein synthesis and, consequently, in the reduction of the body's antioxidant potential. About 50% of individuals of European origin have the *GSTM1*-null genotype, and 10-20% of individuals of European origin and about 50% of Asians have the *GSTT1*-null genotype[44].

Franko *et al.* (2007)[44] evaluated the null polymorphism of *GSTM1* and *GSTT1* in workers exposed to amianthus in Slovenia and observed a significant protective effect of the *GSTT1*-null genotype for asbestosis (OR=0.61; 95% CI 0.40-0.94; P=0.02), even after adjustment for cumulative exposure, smoking, sex and age. However, no association was observed between asbestosis and the *GSTM1*-null genotype (OR=1.01; 95% CI 0.71-1.43; P=0.96). In a study carried out later by the same research group[43], the authors combined the results obtained for the *GSTP1*, *GSTM1* and *GSTT1* genes and did not observe changes in OR values, showing an independent activity and absence of synergistic effect among these genes[43]. In 2021, Franko et al. (2021)[41] evaluated again the association of the same *GSTT1*-null *GSTM1*-null genotypes in another sample from Slovenia and observed the same significant protective effect of the *GSTT1*-null genotype for asbestosis (OR=0.51; 95% CI 0.28-0.93; P=0.028), and no association between asbestosis and the *GSTM1*-null genotype (OR=1.13; 95% CI 0.76-1.69; P=0.721) (Table 1).

Glutathione related enzymes

The glutamate cysteine ligase (GCL) is the rate limiting enzyme of the GSH synthesis and the major factor that determines GSH level in healthy subjects. The enzyme consists of a light modifier subunit (GCLM) and heavy catalytic subunit (GCLC). High GSH concentration levels found in many tumors have been associated with the increased GCL activity[41,45].

The genes coding for GSH related enzymes are polymorphic, but among the most investigated promoter polymorphisms of the *GCLC* and *GCLM* genes are rs17883901 (c.-129C>T) and rs41303970 (c.-590 C>T), respectively. Some studies indicated that polymorphisms in *GCLC* and *GCLM* are associated with low levels of reduced GSH in vitro, which may explain susceptibility to certain diseases related to oxidative stress[41,46]. The *GCLC* rs17883901 polymorphism has been suggested to suppress the *GCLC* gene induction response to oxidants and it has been implicated in several diseases[41,47-49]. Recently, Franko *et al.* (2021)[41] analyzed the association of these *GCL* polymorphisms and asbestosis in 147 patients with asbestosis and 178 exposed healthy controls from Slovenia, and no association was observed (rs17883901: OR=0.95; 95% CI 0.57-1.67; P=0.874, and rs41303970: OR=0.95; 95% CI 0.63-1.43; P=0.369) (Table 1).

Alpha-1-antitrypsin

The alpha-1-antitrypsin (A1AT), is a highly polymorphic anti-elastase enzyme with high activity in alveolar and liver protection. When the asbestos fibers reach the alveolar space, they are phagocytized by the alveolar macrophages and promote the activation of neutrophils, which release several inflammatory mediators, including the elastase (a powerful proteolytic enzyme)[50]. The lower respiratory tract is particularly vulnerable to the deficiency of A1AT, which represents more than 90% of the alveolar anti-

elastases[50,51]. This deficiency could be critical in asbestosis and may contribute to fibrosis of the lung parenchyma in this disease [50]. The *A1AT* gene, located at 14q32.13, shows considerable variability of polymorphic alleles, however, most do not affect the normal concentration levels of the enzyme, with only two alleles considered deficient, Pi*Z (Glu342Lys) and Pi*S (Glu264Val)[50,52].

Lafuente *et al*. (2002)[50] carried out a study of these two alleles in a sample of Spanish individuals exposed to asbestos. The Pi*Z allele was seven times more common in cases (3.5%) than in exposed controls (0.5%) (OR=8.90; 95% CI 1.02-76.40; P=0.04), and the Pi*S allele was twice as frequent in cases than in controls (11.5% versus 6.3%), however this difference was not significant (OR=1.50; 95% CI 0.80-3.05; P=0.10). After multivariate analysis, controlled for age and smoking, the authors observed a significant association between asbestosis and the heterozygous genotype for Pi*Z (OR=8.90; 95% CI 1.02-76.40; P=0.04) and heterozygosity for Pi*Z together with homozygosity for Pi*S (OR=8.00; 95% CI 1.63-39.10; P=0.01). These results suggested that variations in *A1AT*, especially Pi*Z, could be a predictive factor of risk for asbestosis (Table 1).

Catalase

Catalase is responsible for controlling the concentration of H_2O_2 in human cells, through the reduction of H_2O_2 in H_2O and O_2[15]. Several studies suggest that a reduction in its activity may increase the risk of certain diseases, such as asbestosis[15,53,54]. The human gene that encodes catalase (*CAT*) is located on chromosome 11p13 and consists of 13 exons. Several polymorphisms have been reported in *CAT* and the most common one is the replacement of cytosine (C) to thymine (T) at the -262 position of the promoter region of the gene (-262C/T) results[15,53-56]. The relationship between the -262C/T polymorphism and the level of catalase activity has been investigated in several studies, with controversial results[15,53-56].

Franko *et al.* (2008)[15] investigated the influence of the -262 C/T polymorphism and the risk of asbestosis workers exposed to asbestos in Slovenia. The authors observed a slight increase in the odds ratio for asbestosis for the -262TT genotype when compared to combined genotypes CT and CC, however this difference was not statistically significant (OR=1.36; 95% CI 0.70-2,62; P=0.364), even after evaluating the synergistic effect between genotypes and cumulative exposure to asbestos (Table 1).

Interaction between genetic polymorphisms in asbestosis

The analysis of gene-gene and gene-environment interactions more realistically reflect the combined effects of the different genes involved in multifactorial diseases, and, therefore, should be the strategic choice for association studies in this category of diseases.

To evaluate the interactions between different gene polymorphisms and their associations with the development of asbestosis, Franko *et al.* (2013)[57] gathered data regarding the *SOD2*, *SOD3*, *CAT*, *GSTT1*, *GSTM1*, *GSTP1*, and *iNOS* genes from previous publications of their group, in 262 cases and 265 controls from Slovenia. The authors observed an increased risk for the LL genotype of the *iNOS* polymorphism $(CCTTT)_n$ in the presence of the *CAT* -262TT genotype (OR=5.14; 95% CI 1.30-20.36; P<0.0001). The same was observed for the *SOD2* -9Ala/Ala genotype only when the *CAT* -262TT genotype was present (OR=2.67; 95% CI 0.57-13.07; P=0.004), although the OR 95% confidence interval was not significant. In addition, the authors evaluated the effects of polymorphisms on the associations between smoking and asbestosis, and cumulative exposure to asbestos and asbestosis. They concluded that the genotypes *GSTM1*-null and *iNOS* LL modified the risk for asbestosis only in those who smoked (OR=1.48; 95% CI 0.92-2.39; P=0.009; OR=1.39; 95% CI 0.84–2.30; P=0.05, respectively), although the OR 95% confidence intervals were not significant. Regarding cumulative exposure to asbestos, the authors observed that the *iNOS* LL genotype modified the association between cumulative exposure to asbestos (>11.23 fibers/cm3-years) and asbestosis (OR=3.09; 95% CI 1.81-5.25; P<0.0001).

5. CONCLUSION

Asbestosis shows clinical heterogeneity and an average latency time for the development around 20 years[6], and the identification of individual genetic differences that may interfere in the phenotype is progressing very slowly. Besides some studies have observed significant associations between gene variants in *TNFα*, *GSTP1*, *GSTT1*, *SOD2*, and *A1AT* and asbestosis, the limited number of selected studies, carried out by few research groups (3) in only three countries (Slovenia, Germany and Spain), limits extrapolating the results to a global context, since multiple ethnic groups should be analyzed for a better understanding of the impact of genetic variations in different populations. In addition, most polymorphisms were studied individually, and the results have not been replicated in subsequent studies. Only one study evaluated the interactions between genetic polymorphisms and environmental factors and their influence on asbestosis, an approach that would provide results more related to the multifactorial context of the disease.

In conclusion, to further evaluate the impact of gene variations in asbestosis, more studies are still needed. In the future, these studies can bring new perspectives for the patient's follow-up and the development of new classes of anti-fibrotic drugs[58].

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7. DECLARATION ON INTEREST STATEMENT

The authors declared no conflicts of interest.

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