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Review

Genetic susceptibility to malignant pleural mesothelioma and other asbestos-associated diseases

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ABSTRACT

Exposure to asbestos fibers is a major risk factor for malignant pleural mesothelioma (MPM), lung cancer, and other non-neoplastic conditions, such as asbestosis and pleural plaques. However, in the last decade many studies have shown that polymorphism in the genes involved in xenobiotic and oxidative metabolism or in DNA repair processes may play an important role in the etiology and pathogenesis of these diseases. To evaluate the association between diseases linked to asbestos and genetic variability we performed a review of studies on this topic included in the PubMed database. One hundred fifty-nine citations were retrieved; 24 of them met the inclusion criteria and were evaluated in the review. The most commonly studied *GSTM1* polymorphism showed for all asbestos-linked diseases an increased risk in association with the null genotype, possibly linked to its role in the conjugation of reactive oxygen species. Studies focused on *GSTT1* null and *SOD2 Ala16Val* polymorphisms gave conflicting results, while promising results came from studies on α1-antitrypsin in asbestosis and *MPO* in lung cancer. Among genetic polymorphisms associated to the risk of MPM, the *GSTM1* null genotype and two variant alleles of *XRCC1* and *XRCC3* showed increased risks in a subset of studies. Results for the NAT2 acetylator status, *SOD2* polymorphism and EPHX activity were conflicting. Major limitations in the study design, including the small size of study groups, affected the reliability of these studies. Technical improvements such as the use of high-throughput techniques will help to identify molecular pathways regulated by candidate genes.

Contents

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1. Introduction

Exposure to asbestos fibers is a well-known risk factor for malignant pleural mesothelioma (MPM), lung cancer, and other non-neoplastic conditions, such as asbestosis and pleural plaques.

Different mechanisms of damage caused by asbestos fibers have been identified or hypothesized. Inhaled asbestos fibers penetrate the lung epithelium and irritate the pleural cell lining, causing repeated cycles of damage, repair and local inflammation. This repeated scratching may lead to the formation of plaques or to mesothelioma. Another possible mechanism could occur when asbestos fibers interfere with the mitosis. The damages caused to the mitotic spindle could potentially lead to aneuploidy or induce the other typical chromosome anomalies often found in mesothelioma [1].

Asbestos toxicity and carcinogenicity may be mediated by reactive oxygen or nitrogen species (ROS/RNS). This mechanism, activated by the interaction of asbestos fibers with the mesothelial cells and from the prolonged phagocytic activity of inflammatory cells, is probably the most circumstantiated one [1,2]. The free radicals generated by these processes may cause cellular toxicity and carcinogenicity by inducing lipid peroxidation, altering signal transduction pathways, and damaging the DNA directly [3]. Consequences of oxidative damage include single strand breaks and DNA base modification [4]. Furthermore, asbestos-induced DNA damage has been demonstrated to activate tyrosine kinase (TK) both in lung epithelium and in mesothelial cells [5]. In addition, asbestos fibers induce phosphorylation of the mitogen-activated protein kinases and extra cellular signal-regulated kinases 1 and 2 and elevate expression of early response proto-oncogenes (*FOS* or *JUN* or activator protein 1 family members) [6–9].

In the last decade the role of genetic polymorphism in the pathogenesis of cancer and other diseases has been the object of intensive research. Many studies have focused on polymorphic genes active in various steps of xenobiotic and oxidative metabolism, or in DNA repair processes.

Even though mesothelioma has been considered for many years the paradigm of environmentally determined cancers, the presence of a genetic component in the etiology of this disease has been hypothesized, mostly based on the evidence that only a minority of asbestos exposed subjects develop MPM (5–17% of individuals heavily exposed). This consideration, together with the frequent reports of MPM familial clustering [10,11], suggested a role of genetic susceptibility also in this disease. Similar arguments have been carried out also for other asbestos-mediated diseases, both neoplastic, like lung cancer, and non-neoplastic (e.g., asbestosis, pleural plaques).

In this paper, we will review published studies addressing the association between diseases linked to asbestos and genetic polymorphisms. The relevance of genetic factors in explaining the pathogenesis of these diseases will be discussed, with a special focus on MPM.

2. Bibliographic search

The search for papers was performed using the PubMed database (National Library of Medicine, National Institutes of Health, Bethesda, MD, USA—http://www.ncbi.nlm.nih.gov/PubMed) and it was updated up to January 31, 2008. Specific keywords (Mesh terms[mesh]) and free texts terms (words in title or abstract field[tiab]) were used as a search strategy. The first group of terms referred to main concepts related to genetic polymorphisms (polymorphism, genetic[mesh] OR genotype[mesh] as keywords, and polymorphism[tiab] OR polymorphisms[tiab] OR polymorphic[tiab] OR SNP[tiab] OR "single nucleotide polymorphism" as free text in title and in abstract field). The second group referred to main pathologies associated to asbestos exposure (Mesothelioma[mesh] OR (asbestos[mesh] AND (lung neoplasms[mesh] OR pleural neoplasms[mesh] OR pleural diseases[mesh] OR pleura[tiab] OR pleural[tiab])) OR asbestosis[mesh] OR mesothelioma[tiab] OR asbestos[tiab] OR asbestosis[tiab]).

2.1. Inclusion/exclusion criteria

One hundred fifty-nine citations were retrieved through Medline search. All potentially interesting articles were obtained and manually reviewed. Only papers specifically providing a quantitative estimate of the association between genetic polymorphisms and diseases linked to asbestos exposure were further considered. Studies including less than 10 subjects in each study group and studies on animals or *in vitro* were excluded from the analysis. Five potentially interesting articles in Russian and in Chinese could not be translated and were discarded. Twenty-four papers, describing 19 studies, met the inclusion criteria and were reported in the review.

3. Genetic polymorphism and non-neoplastic diseases associated to asbestos exposure

Eight studies evaluated the role of genetic polymorphisms in non-neoplastic diseases associated to asbestos exposure (Table 1). All these studies were conducted in the framework of occupationally exposed subjects (maximum number of subjects: 639 [12,13]). The most common disease was asbestosis, but pleural abnormalities have been investigated as well. Polymorphic genes were genotyped by PCR and restriction enzyme-based methods.

Table 1
Studies on genetic polymorphism in non-neoplastic diseases associated to asbestos exposure

Reference	Country (ethnicity)	Study design	Disease	Cases N exposure	Controls N exposure	Polymorphism	Statistical analysis	Main results (reference is the wild-type genotype)
Jakobsson et al. [17]	Sweden	Cross-sectional	Various chest X-ray abnormalities, impaired lung function	Total 78 asb cement plant workers		*GSTM1* null	Logistic regression adj for age, smoking status, asb exp (linear regression for lung function)	No significant differences between the *GSTM1* groups neither for parenchimal, nor for pleural radiographic abnormalities, nor for lung function
Smith et al., Kelsey et al. [12,13]	USA (95% Caucasians)	Cross-sectional	Asbestosis	55 occ asb exp	584 largely occ asb exp	*GSTM1* null, *GSTT1* null	Logistic regression adj for age, smoking history and occ asb exp	*GSTM1* null OR 2.1 ($p = 0.015$); in non-smokers OR 3.0 ($p = 0.01$). No significant association with *GSTT1* null ($p = 0.85$; crude OR 1.04)
		Cross-sectional	X-ray pleural abnormalities	23 occ asb exp	616 largely occ asb exp	*GSTM1* null, *GSTT1* null	Logistic regression adj for age, smoking history and occ asb exp	No significant association with *GSTM1* null ($p = 0.98$; crude OR 1.3) or *GSTT1* null ($p = 0.39$; crude OR 0.6)
Franko et al. [15]	Slovenia	Nested case–control[a]	Asbestosis	262 asb cement plant workers	265 asb cement plant workers	*GSTM1* null, *GSTT1* null	Logistic regression adj for gender, age, smoking history and cumulative occ asb exp	*GSTM1* null crude OR 1.01 (95% CI 0.7–1.4) *GSTT1* null crude OR 0.6 (95% CI 0.4–0.9). The risk did not change after adj. No synergistic effect of *GSTM1* and *GSTT1 null* genotypes
Hirvonen et al. [14]	Finland	Case–control	Asbestosis and/or pleural plaques	52 highly asb exp insulators	69 highly asb exp insulators	*GSTM1* null, *GSTT1* null, *NAT2* slow/fast acetylator	Crude OR	*GSTM1* null OR 1.5 (95% CI 0.8–3.3). *GSTT1* null OR 0.7 (95% CI 0.2–3.1). *NAT2* slow OR 1.8 (95% CI 0.8–4.2). Combined *GSTM1* null and *NAT2* slow OR 4.1 (95% CI 1.1–17.2)
Horská et al. [16]	Slovakia	Cross-sectional	Asbestosis	27 occ asb exp	34 occ asb exp	*GSTM1* null, *GSTT1* null, *GSTP1 I105V*, *EPHX1*	χ^2	*GSTP1**105Val allele and *EPHX* low activity genotype were protective in exp subjects ($p = 0.048$ and $p = 0.045$, respectively). No effect of *GSTM1* and *GSTT1 null* genotypes
Hirvonen et al. [18]	Finland	Case–control	Asbestosis and/or pleural plaques	41 highly asb exp insulators	63 highly asb exp insulators	*SOD2* Ala16Val	Logistic regression adj for age, asb exp and pack/years of smoking	Ala/Val OR 0.5 (95% CI 0.2–1.4). Val/Val OR 0.8 (95% CI 0.2–2.7). A/V or V/V OR 0.6 (95% CI 0.2–1.5)
Lafuente et al. [20]	Spain	Case–control	Asbestosis	100 occ asb exp	94 occ asb exp	*α1-Antitrypsin Pi*S, Pi*Z*	Logistic regression adj for age and smoking habits	Pi*Z allele freq OR 8.9 (95% CI 1.02–76.4, $p = 0.04$). Pi*S allele freq OR 1.5 (95% CI 0.80–3.1, $p = 0.1$). No subjects were homo ZZ. Homo SS vs. hetero + wt OR 5.9 (95% CI 0.62–56.4, $p = 0.1$)
					121 asb unexp hospital controls	*α1-Antitrypsin Pi*S, Pi*Z*	Crude OR	Pi*Z allele freq OR 2.9 (95% CI 0.67–14.4, $p = 0.1$). Pi*S allele freq OR 1.1 (95% CI 0.6–2.1, $p = 0.6$)
Zhao et al. [19]	China	Case–control	Asbestosis	51 asb plant workers	53 asb plant workers	*XRCC1 Arg399Gln*	Logistic regression adj for gender, age and smoking status	Gln allele genotypes OR 0.95 (95% CI 0.38–2.4)

Methods: PCR, restriction enzymes OR, odds ratio; CI, confidence interval; adj, adjusted or adjustment; occ asb exp, occupationally asbestos exposed or occupational asbestos exposure; unexp, unexposed; freq, frequency; homo, homozygote; hetero, heterozygote; wt, wild-type.

[a] Case–control study nested in a cohort of 2080 occ asb exp workers.

3.1. Polymorphism in genes of xenobiotic metabolism or response to oxidative stress

In a cross-sectional study conducted on a cohort of 639 US carpenters heavily exposed to asbestos, a significant association of *GSTM1* null genotype with asbestosis was reported (OR 2.1, $p = 0.015$; in non-smokers OR 3.0, $p = 0.01$) [12,13]. Odds Ratios for *GSTM1* null genotypes were also increased, but not significantly, in a group of 52 Finnish insulators heavily exposed to asbestos and affected by asbestosis or pleural plaques (OR 1.5, 95% CI 0.8–3.3) [14]. Individuals with *GSTM1* null genotype did not show an increased risk of asbestosis neither in a nested case–control study conducted in Slovenia on 527 asbestos cement plant workers drawn from a cohort of 2080 (OR 1.01) [15], nor in a small Slovakian study [16]. No significant difference was found for X-ray pleural abnormalities depending upon *GSTM1* null genotype both in the US carpenters and in a small group of Swedish workers of an asbestos cement plant [12,13,17].

GSTT1 null genotype was underrepresented in Slovenian asbestosis patients respect to healthy counterparts (OR 0.6, 95% CI 0.4–0.9) [15]. A similar protective effect on the risk of asbestosis was also found in Finnish insulators, although not statistically significant (OR 0.7 for *GSTT1* null subjects) [14]. Similarly, no association could be detected in Slovakian asbestos cement workers and in US [17]. In the US carpenters group the crude ORs were 1.04 for asbestosis and 0.6 for pleural abnormalities, but no significant association could be demonstrated after adjustment for confounding [13].

In addition to the findings reported above, the study performed with Finnish insulators found a lower risk of asbestosis and/or pleural plaques in workers with the NAT2 fast acetylator genotype and in those bearing the *SOD2* Val polymorphism at codon 16. Although the small size of the study did not allow reaching statistical significance [14,18], a 4-fold increased risk was found for subjects with combined NAT2 slow and *GSTM1* null genotypes [14].

In the small Slovakian study *GSTP1*105Val allele and EPHX low activity genotypes were underrepresented in exposed subjects with asbestosis ($p = 0.048$ and $p = 0.045$, respectively) [16].

3.2. Polymorphism in other genes: XRCC1 and α1-antitrypsin

The only evidence concerning the XRCC1 Arg399Gln polymorphism is a report of no association between this variant and the risk of asbestosis in 104 exposed workers from a Chinese asbestos-producing plant [19].

Lafuente et al. [20] evaluated the influence of two polymorphisms in the α1-antitrypsin gene on the risk of asbestosis. The study group included 100 subjects affected by asbestosis and two control groups, 94 workers exposed to asbestos and 121 hospital controls. The product of α1-antitrypsin protects alveolar walls against elastase, a proteolytic enzyme secreted from neutrophils and macrophages, and the authors studied the effect of *Pi*Z* and *Pi*S* deficiency alleles. A higher risk of asbestosis was found in *Pi*Z* heterozygotes when compared to exposed controls working in the same plant (OR = 8.9, 95% CI 1.02–76.4), while the risk was much lower when comparing workers with asbestosis to unexposed hospital controls. However, given the rarity of *Pi*Z* allele (1.2% in unexposed controls; no Pi*Z homozygotes were detected in the whole study group), the contribution of this polymorphism to the overall risk of asbestosis is limited. Pi*S homozygotes showed a 6-fold increased risk of asbestosis, but the limited size of the study group prevented from reaching statistical significance (Pi*S allele frequency: 10% in Spain). According to the authors, only one Russian phenotypic study had been previously conducted on this topic, yielding inconclusive results [21].

4. Genetic polymorphism and exposure to asbestos in lung cancer

Asbestos is a well-known risk factor for lung cancer, although in case–control studies this exposure is frequently not included in the set of risk factors. Eight articles estimating the risk associated to asbestos exposure and the effect modification induced by genetic polymorphism are illustrated in Table 2. The most frequent study design was case–control, but the case-only approach has been used as well. All the genotypes were obtained by PCR and by methods based on restriction enzymes.

4.1. Polymorphism in genes of xenobiotic metabolism

No statistically significant association of *GSTM1* null genotype with lung cancer risk could be demonstrated in a case–control study on 342 lung cancer cases and 716 population controls conducted in the US, although ORs higher than 1.0 were found only in asbestos exposed patients ($n = 142$) [22]. Similarly, a non-significant interaction between *GSTM1* null genotype and asbestos exposure was observed: in a similar case–control study in Finland based on 205 cases (74 exposed to asbestos) and 294 population controls [23]; in a pooled analysis re-evaluating more than 600 lung cancer patients (110 exposed to asbestos); and in a case-only pooled analysis based on 869 patients (189 exposed to asbestos) [24]. In a small Turkish case-only study, the risk of lung cancer was 8-fold higher in *GSTM1* null subjects with history of exposure to carcinogens other than smoking, including asbestos [25].

No interaction between *GSTT1* null polymorphism and asbestos exposure in association with lung cancer was found in a pooled case-only analysis [24].

After stratification by asbestos exposure, the risk of lung cancer in the Finnish study mentioned above was no longer significantly associated with the *NAT2* genotype alone or in combination with *GSTM1* genotypes. However, 13 out of the 16 lung cancer patients who suffered from asbestosis or sub-pleural fibrosis showed the *NAT2* slow acetylator genotype, yielding a 4-fold higher risk with respect to the control population ($p = 0.02$) [23].

In a case–control study of 144 African Americans incident cases, the presence of the *CYP1A1 MspI* variant allele in the whole study group was associated to a 1.3 non-significant risk of lung cancer. The strata specific ORs were 0.8 in unexposed subjects and 2.2 in those possibly exposed to asbestos [26].

Finally, in 1989 Caporaso et al. [27] compared the debrisoquine metabolic phenotype, a trait that was subsequently attributed to *CYP2D6* polymorphisms, in 159 lung cancer and 153 patients with chronic obstructive pulmonary disease, all male smokers. The OR in extensive metabolizers with likely occupational asbestos exposure was 18.4 (95% CI 4.6–74) when compared to poor/intermediate metabolizers with unlikely exposure, with a significant interaction between asbestos exposure and metabolizer phenotype. However, these results have not been replicated yet.

4.2. Polymorphism in genes of response to oxidative stress

In a case–control study with 375 cases and 378 volunteers (about 1/4 asbestos exposed), Schabath et al. [28] studied a polymorphism in the promoter that decreases the expression of *MPO*, a *ROS* generating enzyme. The combination of asbestos exposure and *wt G/G* genotype in the *MPO* promoter yielded an OR of 1.7 (95% CI 1.1–2.7; reference: *G/G* unexposed subjects), while the risk for those with at least one A allele did not differ by asbestos exposure.

A statistically significant increase of lung cancer risk was found in subjects with low exposure to asbestos and with the *SOD2* Val

Table 2
Studies on genetic polymorphism in lung cancer associated to asbestos exposure

Reference	Country (ethnicity)	Study design	Cases N type (N exp)	Controls N type (N exp)	Polymorphism	Statistical analysis	Main results (reference is the wild-type genotype or the unexp in case-only studies)
London et al. [22]	USA (39% African Americans, 61% Caucasians)	Case–control	342 incident lung cancer patients (200 asb unexp)	716 population controls from the files of the Department of Motor Vehicles and of Medicare (470 asb unexp)	GSTM1 null	Logistic regression adj for age, sex, race and lifetime smoking history	No risk variation in *GSTM1* null patients by occ asb exp: All subjects OR 1.3 (95% CI 0.9–1.8); No exp OR 1.03 (95% CI 0.7–1.6); Possible exp OR 1.9 (95% CI 1.03–3.5); Probable exp OR 1.5 (95% CI 0.6–4.2)
Stücker et al. [24]	Pool of studies[a] (various ethnicities)	Pooled case–control	651 lung cancer (110 asb exp)	983 population or hospital controls (127 asb exp)	GSTM1 null	Pooled OR calculated on a fixed effects model, starting from single ORs from unconditional logistic regression adj for age, gender, pack–years of smoking	Pooled OR of interaction *GSTM1* null genotype/asb exp: 1.1 (95% CI 0.6–2.1)
		Pooled case-only	869 lung cancer patients (187 asb exp)	–	*GSTM1 null*		Pooled POR for asb exp and *GSTM1* null 1.2 (95% CI 0.9–1.8)
		Pooled case-only	603 lung cancer (123 asb exp)	–	GSTT1 null		Pooled POR for asb exp and *GSTT1* null 1.1 (95% CI 0.6–2.0)
Saarikoski et al. [23]	Finland (Finnish Caucasians)	Case–control	205 operable lung cancer patients (74 definite or probable asb exp)	294 blood donors (asb exp not assessed)	*GSTM1 null* *NAT2 slow/fast acetylator*	Mantel–Haenszel OR	No statistically significant association with *NAT2*, *GSTM1* or combined genotypes after stratification for asb exp.
		Case-only	16 lung cancer pts with asbestosis or subpleural fibrosis	–	*NAT2 slow/fast acetylator*		Slow acetylator OR 4.0 (95% CI 1.2–13.2)
Ozturk et al. [25]	Turkey	Case–only	55 NSCLC (10 exp to carcinogens other than smoking, including asb)	–	*GSTM1 null*	Crude OR	Patients with *GSTM1* null genotype had 8-fold risk of reporting history of such exp ($p = 0.009$)
London et al. [26]	USA (African Americans)	Case–control	144 incident lung cancer patients	234 population controls as in London 1995a	*CYP1A1 MspI*	Logistic regression adj for age, sex, and lifetime smoking history	All subjects with the variant allele OR 1.3 (95% CI 0.7–2.4). No significant risk variation by occ asb exp: None OR 0.8 (95% CI 0.3–1.9). Possible OR 2.2 (95% CI 0.8–6.1)
Schabath et al. [28]	Usa (Caucasians)	Case–control	375 lung cancer patients (129 occ/non-occ asb exp)	378 healthy volunteers (101 occ/non-occ asb exp)	MPO G or A in the promoter	Logistic regression adj for age, gender, smoking status	G/G asb unexp 1.0 (ref.) G/A + A/A asb unexp OR 0.7 (95% CI 0.5–1.1) G/G asb exp OR 1.7 (95% CI 1.1–2.7) G/A + A/A asb exp OR 0.9 (95% CI 0.6–1.4)
Wang et al. [29]	USA (whites)	Case–control	811 incident lung cancer cases (103 high occ/non-occ asb exp)	957 friends or non-blood relatives of patients (96 high occ/non-occ asb exp)	*SOD2* Ala16Val	Logistic regression adj for age, gender and smoking-related variables	In no-low asb exp subjects: Ala/Ala 1.0 (ref.). Ala/Val OR 1.7 (95% CI 1.3–2.3). Val/Val OR 2.1 (95% CI 1.5–3.0). In high asb exp subjects: no statistically significant association. No statistically significant interaction between asb score and *SOD2* genotype

Table 2 (*Continued*)

Reference	Country (ethnicity)	Study design	Cases N type (N exp)	Controls N type (N exp)	Polymorphism	Statistical analysis	Main results (reference is the wild-type genotype or the unexp in case-only studies)
Caporaso et al. [27]	UK (Caucasians)	Case–control	159 lung cancer patients, male smokers (111 occ asb exp)	153 COPD patients from the same hospitals, male smokers (121 occ asb exp)	Debrisoquine metabolic phenotype (*CYP2D6*)	Logistic regression adj for age, smoking (pack-years) and PAH occ exp	In poor/intermediate debrisoquine metabolizers: No occ asb exp OR 1.0 (ref.). Possible occ asb exp OR 0.6 (95% CI 0.1–3.0). Likely occ asb exp OR 1.8 (95% CI 0.2–19.6). In extensive debrisoquine metabolizers: No occ asb exp OR 6.0 (95% CI 3.0–12.0). Possible occ asb exp OR 8.0 (95%CI 3.3–19.6). Likely occ asb exp OR 18.4 (95% CI 4.6–74). Relative excess risk due to interaction of asb exp and extensive metabolizer phenotype 11.6

Methods: PCR, restriction enzymes, with the exception of Caporaso 1989 (phenotypic assay) OR, odds ratio; CI, confidence interval; adj, adjusted; POR = prevalence odds ratio; COPD = chronic obstructive pulmonary disease (asthma, bronchitis, emphysema); occ asb exp, occupationally asbestos exposed or occupational asbestos exposure; unexp, unexposed.

[a] Includes [22].

allele in a large US study based on 811 incident cases and 957 population controls. The ORs were 1.7 (95% CI 1.3–2.3) in the heterozygotes and 2.1 (95% CI 1.5–3.0) in the homozygotes, respectively. No increase was observed in the group with high exposure to asbestos [29].

5. Genetic polymorphism and MPM

Asbestos exposure is the main risk factor for MPM, a rare and aggressive tumor. MPM is characterized by a poor prognosis and it is scarcely influenced by current therapies, as shown by a median survival from presentation of 9–12 months [1]. Seven published articles report on the risk of MPM in association with genetic polymorphism: three from Finland [14,22,30] and four from Italy [31–34]. The Italian studies were conducted in two areas with high MPM incidence, due to asbestos exposure in local industrial activities. The annual incidence rate of mesothelioma in industrialized countries ranges around 1–2/1.000.000/year for women and 10–30/1.000.000/year for men [35]. In Liguria, where asbestos has been extensively used in shipyard and port activities, the annual incidence in 1996–2002 was 1.43 and 8.51/100.000 for females and males, respectively [36]. In Casale the incidence rate was over 10/100.000 in both men and women, 10-fold higher than in Piedmont general population. Occupational and domestic/environmental exposure to asbestos is frequent in Casale as an asbestos cement factory was active in the area from 1907 to 1985 [37–39].

Results from the largest association studies on MPM and genetic polymorphism are presented according to the geographic area were the studies were carried out (Table 3).

5.1. Finnish studies: polymorphism in genes of xenobiotic metabolism or response to oxidative stress

The first association studies on MPM were conducted in Finland in mid 90s and they focused on few metabolic genetic polymorphisms, analyzed with simple techniques based on PCR and restriction enzymes. A group of 44 mesothelioma patients (epithelial, mixed or fibrous histology) were compared to 270 blood donors [30]. Data were analyzed using the logit model. Both the *GSTM1* null and *NAT2* slow acetylator almost doubled the risk of MPM (OR 1.8, 95% CI 1.0–3.5, $p = 0.06$, and OR 2.1, 95% CI 1.1–4.1, $p = 0.03$, respectively). The OR for those with combined *GSTM1* null and *NAT2* slow acetylator genotypes was 3.6 (95% CI 1.3–10.2, $p = 0.006$) when compared to subjects with the combination of functional *GSTM1* and *NAT2* fast acetylator. Risks were higher in the subset of 24 patients that had experienced the highest asbestos exposure (up to OR 7.4, 95% CI 1.6–34.0, $p = 0.002$ for the combined genotypes). The latter 24 subjects were subsequently compared to a second group of controls, including 69 asbestos insulators with no pulmonary disorder at chest X-rays, with the aim of analysing the effect of genotypes when similar levels of asbestos exposure were present [14]. The risk calculated with the Fisher's exact model for *GSTM1* null was more than double, but not statistically significant, while for *NAT2* slow acetylators the OR was 3.8 (95% CI 1.2–14.3). The combination of both unfavourable genotypes yielded an OR = 7.8 (95% CI 1.4–78.7) in MPM patients, and OR = 4.1 (95% CI 1.1–17.2) in 52 patients suffering from asbestosis and/or pleural plaques, when compared to healthy insulators. No significant risk was associated with *GSTT1* null genotype.

Since MnSOD (protein encoded by *SOD2* gene) activity is elevated in MPM biopsies [40,41], in 2002 Hirvonen et al. [18] investigated whether the *SOD2* Ala16Val polymorphism modified individual susceptibility to MPM or to non-malignant asbestos-associated pulmonary disorders. This study selected 61 cases

Table 3
Overview of association studies on genetic polymorphism in MPM

Genotypes	Finnish studies[a] OR (95% CI)	Italian studies[b] OR (95% CI)
CYP1A1 Msp1 homo wt	1.0	1.0
hetero + homo variant	1.7 (0.6–4.9)	1.1 (0.6–2.1)
GSTM1 functional	1.0	1.0
Null	1.8 (1.0–3.5)	1.7 (1.04–2.7)[c]
GSTT1 functional	1.0	1.0
Null	1.3 (0.4–3.9)	1.3 (0.7–2.4)
NAT2 slow acetylator	2.1 (1.1–4.1)[d]	1.0
Fast acetylator	1.0	1.7 (1.02–3.0)[e]
SOD2 V16A Val/Val	2.0 (0.3–11.9)	1.0
Ala/Val	1.4 (0.3–6.3)	1.0 (0.5–2.0)
Ala/Ala	1.0	3.1 (1.6–6.1)[f]
EPHX high activity	1.0	1.0
Intermediate activity	0.6 (0.2–1.8)	2.2 (0.96–4.9)
Low activity	0.7 (0.2–2.3)	2.5 (1.11–5.7)
LRT for trend		$p < 0.04$[g]
XRCC1 R399Q homo wt		1.0
hetero + homo variant		2.2 (1.08–4.3)
XRCC3 T241M homo wt		1.0
hetero + homo variant		4.1 (1.3–13.2)
GSTA2 T111S, *GSTA4 rs1802061*; *rs405729*, *GSTM1 a/b*, *GSTM3 del*(*3bp*), *GSTP1 I105V*, *A114V*, *XRCC1 R194W*, *XRCC3 IVS6-14*, *XPD K751Q*, *D312N*, *OGG1 S326C*		No statistically significant association

OR, odds ratio; CI, confidence interval; LRT, likelihood ratio test; homo, homozygote; hetero, heterozygote; wt, wild-type.
[a] Finnish data are reported from Hirvonen et al. [30] for GSTM1 and NAT2; Hirvonen et al. [18] for SOD2; Neri et al. [32] for CYP1A1, GSTT1 and EPHX.
[b] Italian data are reported from Neri et al. [31] for CYP1A1, NAT2, EPHX; Landi et al. [34] for the GSTs and SOD2; Dianzani et al. [33] for the DNA repair genes.
[c] $p = 0.03$.
[d] In high asbestos exposure subjects OR 3.8 (95% CI 1.2–14.3) [14].
[e] Patients with high asbestos exposure OR 2.1 (95% CI 1.2–4.0) [31].
[f] $p = 0.001$.
[g] Patients with low asbestos exposure: intermediate activity OR 6.6 (95% CI 0.8–53.0), low activity OR 7.8 (95% CI 0.98–62.6), LRT for trend $p < 0.04$ [31].

(20 MPM affected and 41 non-malignant diseases affected) and 63 controls with the same asbestos exposure. The study did not reveal any significant association between the *SOD2* genotypes and individual susceptibility to MPM or to non-malignant pulmonary disorders.

5.2. Italian studies: polymorphism in genes of xenobiotic metabolism or response to oxidative stress

GSTM1, *GSTT1* and *NAT2* polymorphisms were analyzed by PCR and restriction enzyme-based techniques in 80 MPM patients living in Liguria and compared with a group of 255 healthy volunteers [31,32]. Logistic regression modelling was applied to assess the joint predictive role of polymorphisms investigated on the disease. In agreement with Finnish data, the *GSTM1* null genotype showed an increased risk, although not statistically significant (OR 1.48, 95% CI 0.86–2.54), while no risk was associated with the *GSTT1* null genotype. When MPM patients were divided into two categories, i.e., subjects with a high probability of asbestos exposure and subjects with a low probability, no remarkable difference in the risk was found. In contrast with Finnish studies, the *NAT2* genotype was associated with an increased risk for the fast acetylator, i.e., OR = 1.7 (95% CI 1.02–3.0) for the whole group of patients, and OR = 2.1 (95% CI 1.2–4.0) in the more likely exposed subjects.

Two more polymorphisms, *CYP1A1* and *EPHX*, were assessed in the same Italian group and in the framework of a collaborative study, in the group of highly exposed Finns studied by Hirvonen et al. [18,32]. As regards to *EPHX*, all subjects were classified as low, intermediate and high activity, according to the *Tyr113His* and *His139Arg* polymorphisms. The low and the intermediate activity genotypes showed an increased risk in the Italian study group (statistically significant for low activity; p for trend <0.04). The risk appeared to be entirely driven by the 23 subjects with low probability of asbestos exposure (OR 7.8, 0.98–62.6 for low activity, p for trend <0.04). On the other hand, in the Finnish group of highly exposed subjects the ORs for low and intermediate EPHX activity were non-significantly decreased. *CYP1A1 MspI* was not associated with MPM risk in any group. When the combination between *NAT2* and *EPHX* genotypes was examined, remarkable differences appeared between the two study groups. In the Italian group, the *NAT2* fast acetylator genotype and the *EPHX* low activity genotype were positive risk factors with a clear synergistic effect (OR 3.5, 95% CI 0.9–13.8, p for trend = 0.007), whereas in the Finns this genotype combination appeared as a protective factor (OR 0.6, 95% CI 0.1–2.7, p for trend = 0.06).

The different impact on the MPM risk of polymorphic alleles of glutathione-*S*-transferases (*GSTs*) and manganese superoxide dismutase (*SOD2*) genes involved in the defence against oxidative damage was investigated using new array-based genotyping techniques [42,34]. Ninety cases of MPM from Liguria and 395 controls were included in this study. Logistic regression analysis was applied to evaluate the statistical association between each polymorphism and risk of MPM, after adjustment for potential confounders. An increased risk of MPM was found in subjects bearing a *GSTM1* null allele (OR 1.7, 95% CI 1.04–2.7, $p = 0.03$), and in those with the Ala/Ala genotypes at codon 16 within *SOD2* (OR 3.1, 95% CI 1.6–6.1, $p = 0.001$). A stronger effect of *SOD2* was

observed among patients without a clear exposure to asbestos fibers. No effect was found for *GSTA2*, *GSTA4*, *GSTM3*, *GSTP1*, and *GSTT1* genetic polymorphisms.

5.3. Italian studies: polymorphism in DNA repair genes

Another Italian study was conducted in the population of Casale Monferrato (Piedmont), addressing the hypothesis that an imperfect DNA repair, as revealed by subtle polymorphic variants, could reduce protection against the chronic DNA insult caused by asbestos. A study conducted with restriction enzyme digestion and primer extension-based techniques on seven variants in four DNA repair genes revealed an association between MPM and SNPs in *XRCC1* and *XRCC3* [33]. This study included 81 MPM patients and 110 age- and sex-matched controls, all from Casale Monferrato. Unconditional multivariable logistic regression was used. When considered as a categorical variable, the *XRCC1 R399Q* variant showed borderline increased risks both in heterozygotes (OR 2.1, 95% CI 1.0–4.3) and homozygotes (OR 2.4, 95% CI 0.8–6.9), when considered as a continuous variable (codominant model), a significant association (OR 1.7, 95% CI 1.02–2.8) was found. When genotypes were divided into "non-risk" and "risk" according to the functional significance of the variants, *XRCC1 R399Q* (Q homozygotes + Q/R heterozygotes vs. R homozygotes) had an OR = 2.1 (95%CI 1.08–4.3), *XRCC3 T241M* (T homozygotes + M/T heterozygotes vs. M homozygotes) had an OR = 4.1 (95% CI 1.3–13.2), while the OR of *OGG1 S326C* was increased, though not significantly. Two SNPs for each of the three genes, i.e., *XRCC1*, *XRCC3*, and *XPD*, were studied, and the haplotype association with MPM was calculated. However, none of the haplotypes showed a significantly different frequency between patients and controls.

6. Biorepositories as a tool for studying genetic susceptibility to MPM: two Italian experiences

Biomarker studies require processing and storage of numerous biological samples with the goals of obtaining a large amount of information and minimizing future research costs. An efficient study design includes original samples processing provisions, such as cryopreservation, DNA isolation, and specimens preparation for subsequent analysis. This approach is necessary especially when the studied condition is rare and biological samples are collected over a long period of time. To address the specific need of studies on MPM and obtain the quality that gives biospecimens their long-term value, two biorepositories for molecular epidemiology studies have been set up in the high-risk Italian areas briefly described below.

6.1. CREST biorepository

The CREST (Cancer of Respiratory Tract) biorepository has been established in 1996 within the National Cancer Research Institute of Genoa. The main objective of this initiative was to support the conduction of multi-centric molecular epidemiology studies of lung cancer and MPM. Biological specimens are collected from incident cases recruited in pneumology departments of major general hospitals located in the region. Three types of controls are also recruited, i.e., healthy subjects (blood donors, recreational associations), controls hospitalized for non-neoplastic, non-respiratory conditions (mostly traumatic diseases or eye diseases), and controls hospitalized for non-neoplastic respiratory conditions (mostly COPD and asbestosis). Subjects are recruited after active search, and after informed consent is obtained, peripheral blood samples are collected with coded vacutainers by routine venipuncture. When blood drawing is not possible, as an alternative the saliva is collected in Oragene vials for DNA extraction. Several different biological samples are preserved: whole blood, plasma, serum, lymphocytes and, whenever possible, pleural fluid and bioptic or surgery cancer tissue samples. The overall number of subjects recruited as of January 2008 is 1700 (including 210 MPM and 440 lung cancer).

6.2. Casale biorepository

A population based biorepository has been established within the district of Casale Monferrato to conduct case-control studies on MPM and to assess the risk associated with environmental asbestos exposure and the interaction of this exposure with genetic polymorphism [37,38]. All residents with an histologically confirmed diagnosis of MPM during the study period (started in January 2001) are considered for the inclusion in the biorepository. Two controls per patient, matched for sex and age, are randomly sampled from the resident population using the rosters made available from the Local Health Authority. Cases are first informed about the study during their stay at the hospital, and then interviewed at home or during subsequent visits. All subjects included in the biorepository are interviewed on their lifelong occupational and residential history by a trained interviewer using a validated questionnaire [38]. Before the interview, an informed consent statement is signed by all participants. Blood samples are obtained from patients and controls (response rate 75% and 44%). To evaluate asbestos exposure, the questionnaires are reviewed by an industrial hygienist who blindly assesses likelihood, intensity and frequency of asbestos exposure due to occupational, environmental and household circumstances. Two hundred MPM cases and 220 controls have been recruited as of May, 2007. DNA samples from all of them have been extracted and are stored in the DNA bank. Sera and frozen vital cells are also available and have been used for studies on SV40 virus exposure.

7. Discussion

Although the role of asbestos as a direct cause of MPM, lung cancer, asbestosis and other respiratory tract diseases is well established, it has been hypothesized that the effect of asbestos exposure may be modified by genetic polymorphisms, and a number of studies explored this issue.

7.1. Genetic polymorphism in asbestos-linked cancers and non-neoplastic diseases

GSTM1 null genotype is likely to play a role in asbestos-related diseases, as shown by the consistent increase of risks found in all published studies, especially in the group of highly exposed subjects. Statistical significance was reached in two studies on lung cancer [22,25] and in two on MPM [30,34], but not in a pooled analysis on lung cancer [24; including data from 22]. Results concerning non-neoplastic diseases are compatible with a role of this polymorphism, although the study with the largest study group did not find any effect [15] and only one study shows a significantly increased risk of asbestosis in *GSTM1* null subjects [12,13]. The biological plausibility of this association is quite strong, considering the role of *GSTM1* in the ROS conjugation, an important step in cellular response to oxidative damage.

Studies on the *GSTT1* null polymorphism showed inconsistent results, suggesting a limited role of this genotype in the pathogenesis of asbestos-related diseases. *GSTT1* null genotype showed a protective effect, if any, for non-neoplastic asbestos-linked diseases (statistically significant only in one study [15]), but in parallel slightly increased the risk of MPM. *GSTT1* null genotype

was underrepresented in cases also in a pooled analysis of 272 lung cancer patients and 323 controls, all asbestos exposed [48].

GSTT1 null genotype was underrepresented in cases also in a pooled analysis of 272 lung cancer patients and 323 controls, all asbestos exposed [S. Raimondi, V. Paracchini, H. Autrup, J.M. Barros-Dios, S. Benhamou, P. Boffetta, M.L. Cote, I.A. Dialyna, V. Dolzan, R. Filiberti, S. Garte, A. Hirvonen, K. Husgafvel-Pursiainen, E.N. Imyanitov, I. Kalina, D. Kang, C. Kiyohara, T. Kohno, P. Kremers, Q. Lan, S. London, A.C. Povey, A. Rannug, E. Reszka, A. Risch, M. Romkes, J. Schneider, A. Seow, P.G. Shields, R.C. Sobti, M. Sørensen, M. Spinola, M.R. Spitz, R.C. Strange, I. Stücker, H. Sugimura, J. To-Figueras, S. Tokudome, P. Yang, J.M. Yuan, M. Warholm, E. Taioli, Meta- and pooled analysis of GSTT1 and lung cancer: a HuGE-GSEC review, Am. J. Epidemiol. 164 (2006) 1027–1042].

The few studies focused on *SOD2* Ala16Val polymorphism, although supported by a strong biological rationale, gave conflicting results. Other genetic polymorphisms have been analyzed in single studies and therefore they cannot be properly evaluated. Nevertheless, interesting suggestions for future studies came from one study on the role of the relatively rare *Pi*S* and *Pi*Z* alleles of α1-antitrypsin in asbestosis [20] and from one study on *MPO*, an enzyme that is abundant in neutrophils and generates ROS: the wild-type genotype, characterized by high *MPO* expression, is associated with high-risk of lung cancer in asbestos exposed subjects [28].

7.2. Focus on MPM

Published data on MPM come from a total number of less than 250 patients from three study areas. The rarity of MPM makes this number remarkable, albeit still too limited for extensive genomic analyses. No association with MPM risk has been consistently demonstrated for polymorphic genes involved with metabolism or DNA repair. However, some associations have been found involving various SNPs in *XRCC1* and *XRCC3*, which deserves further attention. The *GSTM1* null genotype showed a borderline increased risk studies from Finland and Liguria, while *GSTT1* null and *CYP1A1 MspI* polymorphisms were associated with slight non-significantly increased risk. Interestingly, Finnish and Italian populations showed an association between MPM and the same polymorphisms in *SOD2*, *EPHX* and *NAT2*, though in the opposite direction. Italians and Finns differ in terms of genetic background, diet, lifestyle, and type of asbestos predominantly used. However, given the small numbers of genotyped subjects, these conflicting findings can also be simply attributed to chance, as discussed in Neri et al. [32]. The different ethnicity may suggest that the association is not with the studied polymorphisms, but with other genetic variants in linkage disequilibrium with them. In this case the polymorphisms highlighted by these studies would represent true modifiers of the susceptibility in both populations, possibly acting through different polymorphic genes.

7.3. Heterogeneity and other limitations of the studies

Several sources of heterogeneity can be identified in the studies reviewed. For instance, there are remarkable differences in exposure assessment and definition. In most studies only occupational exposure is accounted for (according to job title, specific task, etc.), while environmental or domestic exposure is hardly considered. Furthermore, exposure is generally categorized in 2 or 3 rough levels (sometimes scores for intensity or frequency of exposure are used), while more comprehensive job-exposure matrices are rarely used. Sometimes asbestos exposure is evaluated only in cases and not in controls. Studies markedly differ with respect to controls, selected from volunteers, healthy subjects working in the same plant and sharing the same exposures, or from patients hospitalized for unrelated causes.

From a technical viewpoint, different laboratories used different techniques and different primers, and this may result in different sensitivity and specificity (for example, the Taqman technology has slightly greater sensitivity and specificity than PCR-RFLP). However, it should be noted that all methods of genotyping adopted by the different laboratories have been largely validated, and can hardly justify the contrasting association described above.

A major limitation of the studies included in the review is the small sample size, especially when MPM or non-neoplastic diseases are concerned. Numbers are higher in lung cancer studies where, however, the most significant results are often based on small subgroups of subjects. This weakness creates a problem of statistical power. Furthermore, when other carcinogens are involved, such as tobacco smoke in lung cancer, it may be difficult to disentangle the role of asbestos. Most studies on lung cancer could not efficiently evaluate if the polymorphism investigated exerted its effect modifying the effect of asbestos exposure or the effect of cigarettes smoking. This was mostly due to the small study groups, but also to the poor study design, and the presence of a common pathway based on the oxidative damage for both carcinogens. A detailed evaluation of the relationship between *MPO* polymorphism, asbestos exposure and tobacco smoking was done by Schabath et al. who reported an independent effect of the A-allele genotypes on both exposures, although the protective effect on subjects exposed to asbestos was more evident.

Another limitation affecting association studies is the presence of multiple comparisons, particularly when using microarray platforms that allow the simultaneous genotyping of many SNPs in the same sample. When many tests are performed, false-positive results are expected, although this may be acceptable for an exploratory study [43]. However, when a large number of tests are performed, it is generally advisable to adopt some correction for multiple comparisons, in an attempt to reduce the rate of false-positive findings. Recently, a method for evaluating the probability to get a false-positive result by applying the Bayesian approach has been proposed by Wacholder et al. [44]. This method requires the estimation (from existing knowledge) of the prior probabilities that specific SNPs are associated with the disease under study. This kind of correction was used in Dianzani et al. [33] and in Landi et al. [34].

8. Future directions

In conclusion, although the published studies are based on relatively low numbers of subjects, their results suggest that some genetic polymorphisms may modify the risk of MPM and other neoplastic and non-neoplastic diseases caused by exposure to asbestos fibers. In particular, the evidence from polymorphisms of genes involved in oxidative stress and DNA repair seems more circumstantiated. Studies in larger panels of patients and controls are necessary to confirm preliminary data.

Nowadays, recent advances in high-throughput techniques allow to genotype a large number of SNPs. Thus, it is now possible to perform exploratory studies at whole-genome level, generally known as genome-wide association studies or GWAS. These studies are mostly hypothesis-generating, however, they have the potential to identify a set of empirical tagging SNPs (tSNPs) that best capture the common genetic variation within the genes. They serve as markers to detect associations between a particular region and diseases, whether or not the tSNPs themselves have a functional effect [45]. The tagged approach is extremely efficient, since the alleles of SNPs that are in physical proximity tend to be correlated, i.e., they are in linkage disequilibrium [46]. The

HapMap online database (http://www.hapmap.org) allows the application of the tagging approach to many genes or regions [47].

The long-term goal, besides the obvious improvement in the exposure assessment, will be the integration of data from differentially expressed genes and genomic polymorphisms in a biologically meaningful context. This approach will allow identifying those molecular pathways regulated by candidate genes which affect individual risk, and will help to efficiently address preventive policies in exposed populations.

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