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Pooled analysis of NAT2 genotypes as risk factors for asbestos-related malignant mesothelioma

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Abstract

Malignant mesothelioma (MM) is a rare and aggressive tumor of the pleura. The most important causal factor for the development of MM is occupational exposure to asbestos. Different lines of evidence suggest a role of genetic background in MM development, as for other cancers. Two published studies observed an association between MM and *N*-acetyl-transferase 2 (NAT2) polymorphisms. First, a Finnish study observed that the NAT2 slow acetylator phenotype was associated with an increased risk of MM. Conversely, MM risk was higher in Italian subjects carrying the NAT2 fast acetylator genotypes. The conflicting results obtained in Finland and Italy could be ascribed to random chance, considering the small panel of patients and controls in the two studies, but also ethnic or other differences may have been important. To ascertain the role of NAT2 genotype, we performed a study on 252 MM patients and 262 controls recruited in two Northern Italy areas that were characterized by high asbestos exposure, due to intense industrial activities (an asbestos cement factory in Casale Monferrato, mainly shipyards and refineries in Liguria). Unconditional multivariate logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). NAT2 fast acetylator genotypes showed an increased OR, although not statistically significant, both in asbestos-exposed subjects (OR = 1.47; 95% CI = 0.96–2.26) and in the entire population (OR = 1.38; 95% CI = 0.93–2.04). These results suggest that NAT2 polymorphisms do not exert a strong effect on individual susceptibility to MM.

Keywords: Asbestos; Mesothelioma; *N*-acetyltransferase; Oxidative stress; Single nucleotide polymorphism

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Introduction

The association of asbestos exposure and malignant mesothelioma (MM) is well documented, but the mechanism of action has not been completely clarified (Robinson et al., 2005). Asbestos fibers chronically retained in the lung and the pleura can be carcinogenic as the result of mechanical effects, such as interference with mitotic spindle formation and the segregation of chromosomes, leading to breaks and aberrations (Jensen et al., 1996). A different hypothesis involves the generation of reactive oxygen species (ROS) either by reactions promoted by catalytic iron ions contained in asbestos molecules or by frustrated fagocytes (Chen et al., 1996; Fung et al., 1997). Asbestos-induced oxidative damage has been clearly demonstrated, both in vitro and in vivo: its consequences include DNA single-strand breaks and DNA base modification (Wang et al., 1998). A promotion effect is also suggested by the effect of asbestos exposure on the induction of signal transduction and methylation of gene promoters (Lee and Testa, 1999; Manning et al., 2002; Yang et al., 2006; Zanella et al., 1996; Christensen et al., 2008).

About 80% of MM patients have a history of asbestos exposure (Robinson et al., 2005), but only 5–10% of individuals exposed to high levels actually develop MM and its onset is usually 30–40 years after the first exposure (Bianchi et al., 1997). The combined role of genetics and asbestos exposure, suggested by familial aggregation (Ascoli et al., 1998; Roushdy-Hammady et al., 2001), has been discussed recently in two extensive literature reviews (Ascoli et al., 2007; Ugolini et al., 2008).

Several genetic association studies addressed the identification of the traits that may predispose to asbestos damage susceptibility and MM (reviewed in Neri et al., 2008). Most studies so far have addressed specific hypotheses, under the candidate gene approach. An association of gene polymorphisms leading to defective DNA repair with an increased risk of MM has been explored, for the first time, in the framework of a population-based case–control study conducted in Casale Monferrato, a Northern Italian area subjected to heavy exposure due to an important asbestos cement factory (Dianzani et al., 2006). An association of *XRCC*1-399Q and *XRCC*3-241 T with the risk of MM was observed.

Other studies were focused on genes involved in xenobiotic metabolism and in protection from ROS, under the hypothesis that a reduced protection might increase DNA damage and facilitate carcinogenesis. A group of subjects from the Cancer of RESpiratory Tract (CREST) biorepository in Liguria (Northern Italy), where asbestos has been extensively used in port, shipyard and industrial activities, showed an increased risk of MM in subjects bearing a GSTM1 null allele and in those with the Ala/Ala genotypes at codon 16 within MnSOD (Landi et al., 2007).

Controversial results were found regarding MM risk associated to *N*-acetyltransferase 2 (*NAT*2). Hirvonen et al. (1995, 1996) showed an increased risk of MM in Finnish subjects occupationally exposed to asbestos and carrying the *NAT*2 slow acetylator phenotype, whereas Neri et al. (2005) found an increased risk for *NAT*2 fast acetylator in CREST subjects. The latter study also reported an association of MM with microsomal Epoxide Hydrolase (mEH) and found that *mEH* interacted with both *NAT*2 and *GSTM*1 genes according to a multiplicative model. The *GSTM*1 null genotype showed an increased risk in studies from both Finland and Liguria (Neri et al., 2006).

The discrepancies among the studies of Hirvonen and Neri could be due to chance, but the different ethnic origin and habits of the two studied populations, the Finns and the Italians, could have a role. A main drawback of these studies was the low number of cases and controls.

NAT2 phenotypes have been associated with other cancer types, such as bladder cancer (Sanderson et al., 2007; Vineis, 2002): in this case the biological effect is expected to be mediated by exposure to carcinogenic aromatic amines. The carcinogenic mechanism favoured by these SNPs in MM is unclear.

In this paper, we evaluated NAT2 genotypes in two studies conducted in Northern Italy: a panel of 133 cases and 182 controls from the case–control study in Casale Monferrato and 119 patients and 80 controls from Liguria CREST biorepository. The pooled analysis of both datasets was carried on.

Patients and methods

Study population: the Casale Monferrato panel

A population-based case–control study on MM of the pleura was conducted within Casale Monferrato Local Health Authority (LHA) to assess the risk associated with environmental asbestos exposure and the interaction of such exposure with genetic polymorphisms. Asbestos exposure (both occupational and domestic/environmental) was frequent because an asbestos cement factory had been active in Casale from 1907 to 1985 (Magnani et al., 2000, 2001, 2007).

This study involved the cases of MM histologically diagnosed during the study period (from January 2001 to December 2005) in subjects resident in Casale Monferrato LHA. Routine histology included immunohistochemical staining. Histological types of patients are reported in Table 1.

The study design has been reported in detail elsewhere (Dianzani et al., 2006). Briefly, controls were randomly sampled from the local population using the rosters