Genetic Variants Associated with Increased Risk of Malignant Pleural Mesothelioma: A Genome-Wide Association Study

Giuseppe Matullo1,2, Simonetta Guarrera1, Marta Betti3, Giovanni Fiorito1, Daniela Ferrante4, Floriana Voglino1, Gemma Cadby5,6,7, Cornelia Di Gaetano1,2, Fabio Rosa1, Alessia Russo1,2, Ari Hirvonen8, Elisabetta Casalone3, Sara Tunesi4, Marina Padoan4, Mara Giordano9, Anna Aspesi9, Caterina Casadio10, Francesco Ardissone11, Enrico Ruffini12, Pier Giacomo Betta13, Roberta Libener13, Roberto Guaschino14, Ezio Piccolini15, Monica Neri16, Arthur W. B. Musk17,18, Nicholas H. de Klerk19, Jennie Hui18,20, John Beilby18,20, Alan L. James17,18, Jenette Creaney17,18, Bruce W. Robinson17,18, Sutapa Mukherjee21,22, Lyle J. Palmer5,6, Dario Mirabelli23,24, Donatella Ugolini25, Stefano Bonassi16, Corrado Magnani4,24, Irma Dianzani3,24*

1 Human Genetics Foundation, HuGeF, Turin, Italy, 2 Department of Medical Sciences, University of Turin, Turin, Italy, 3 Laboratory of Genetic Pathology, Department Health Sciences, University of Piemonte Orientale, Novara, Italy, 4 CPO-Piemonte and Unit of Medical Statistics and Epidemiology, Department Translational Medicine, University of Piemonte Orientale, Novara, Italy, 5 Genetic Epidemiology and Biostatistics Platform, Ontario Institute for Cancer Research, Toronto, Ontario, Canada, 6 Prosserman Centre for Health Research, Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada, 7 Centre for Genetic Epidemiology and Biostatistics, University of Western Australia, Nedlands, Western Australia, Australia, 8 Centre of Expertise for Health and Work Ability, Finnish Institute of Occupational Health, Helsinki, Finland, 9 Laboratory of Genetics, Department Health Sciences, University of Piemonte Orientale, Novara, Italy, 10 Thoracic Surgery Unit, University of Piemonte Orientale, Novara, Italy, 11 Chest Surgery, Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy, 12 Thoracic Surgery Unit, University of Turin, Turin, Italy, 13 Pathology Unit, Azienda Ospedaliera Nazionale SS, Antonino e Biagio e Cesare Arrigo, Alessandria, Italy, 14 Transfusion Centre, Azienda Ospedaliera Nazionale SS, Antonino e Biagio e Cesare Arrigo, Alessandria, Italy, 15 Pneumology Unit, Sant’Anna Hospital, Casale Monferrato, Italy, 16 Unit of Clinical and Molecular Epidemiology ERCCS San Raffaele Pisana, Rome, Italy, 17 Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia, 18 National Centre for Asbestos Related Disease, School of Medicine and Pharmacology, University of Western Australia, Nedlands, Western Australia, Australia, 19 Centre for Child Health Research, The University of Western Australia, Nedlands, Western Australia, Australia, 20 PathWest Laboratory Medicine WA, Nedlands, Western Australia, Australia, 21 Department of Medicine, University of Toronto, Toronto, Ontario, Canada, 22 Women’s College Research Institute and Women’s College Hospital, Toronto, Ontario, Canada, 23 Unit of Cancer Epidemiology, CPO-Piemonte and University of Turin, Turin, Italy, 24 Interdepartmental Center for Studies on Asbestos and other Toxic Particulates “G. Scarselli”, University of Turin, Turin, Italy, 25 Department of Internal Medicine, University of Genoa and IRCSS AOI San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Abstract

Asbestos exposure is the main risk factor for malignant pleural mesothelioma (MPM), a rare aggressive tumor. Nevertheless, only 5–17% of those exposed to asbestos develop MPM, suggesting the involvement of other environmental and genetic risk factors. To identify the genetic risk factors that may contribute to the development of MPM, we conducted a genome-wide association study (GWAS: 370,000 genotyped SNPs, 5 million imputed SNPs) in Italy, among 407 MPM cases and 389 controls with a complete history of asbestos exposure. A replication study was also undertaken and included 428 MPM cases and 1269 controls from Australia. Although no single marker reached the genome-wide significance threshold, several associations were supported by haplotype-, chromosomal region-, gene- and gene-ontology process-based analyses. Most of these SNPs were located in regions reported to harbor aberrant alterations in mesotheloma (SLC7A14, THRBB, CEBP350, ADAMTS2, ETV1, PVT1 and MMP14 genes), causing at most a 2–3-fold increase in MPM risk. The Australian replication study showed significant associations in five of these chromosomal regions (3q26.2, 4q32.1, 7p22.2, 12q11.2, 15q14). Multivariate analysis suggested an independent contribution of 10 genetic variants, with an Area Under the ROC Curve (AUC) of 0.76 when only exposure and covariates were included in the model, and of 0.86 when the genetic component was also included, with a substantial increase of asbestos exposure risk estimation (odds ratio, OR: 45.28, 95% confidence interval, CI: 21.52–95.28). These results showed that genetic risk factors may play an additional role in the development of MPM, and that these should be taken into account to better estimate individual MPM risk in individuals who have been exposed to asbestos.

Citation: Matullo G, Guarrera S, Betti M, Fiorito G, Ferrante D, et al. (2013) Genetic Variants Associated with Increased Risk of Malignant Pleural Mesothelioma: A Genome-Wide Association Study. PLoS ONE 8(4): e61253. doi:10.1371/journal.pone.0061253

Editor: Xiao-Ping Miao, MOE Key Laboratory of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, China

Received January 7, 2013; Accepted March 6, 2013; Published April 23, 2013

Copyright: © 2013 Matullo et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by the Regione Piemonte Ricerca Sanitaria Finalizzata 2007, 2008, 2009 (to I.D.), Fondazione Buzzi Unicem Onlus 2007 (to I.D., S.B), CIFE (to I.D.), AIRC (to I.D., D.U., S.B) and Human Genetics Foundation - HuGeF (to G.M.). The Turin case-control study was supported by a grant from Regione Piemonte, Ricerca Scientifica Applicata 2003 (to D.M.). The Casale case-control study was supported by a grant from Regione Piemonte, Ricerca Sanitaria Finalizzata 2004 (to C.M.). The Australian studies have been supported by the Australian National Health and Medical Research Council, the Sir Charles Gairdner Hospital and PathWest laboratory Medicine of WA. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors declare no competing financial interest. In fact, the “PathWest Laboratory Medicine WA” is not a commercial funder of this research. The authors Jennie Hui and John Beilby are employed by PathWest and do not have any additional consultancy, patents, products in development or marketed products with competing interests relating to this research. Thus, PathWest affiliation does not alter the authors’ adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: giuseppe.matullo@unito.it (GM); irma.dianzani@med.unipmn.it (ID)
Introduction

Malignant pleural mesothelioma (MPM) is a rare, aggressive tumor that generally causes death within 2 years. The only clearly established risk factors for MPM are asbestos exposure, and exposure to erionite, other mineral fibers and x-ray for medical purposes [1]. Asbestos fibers retained in the lung and pleura may be carcinogenic, either through direct mechanical or biochemical effects, or through the activation of inflammatory cells. Persistent inflammation can induce chronic oxidative stress, genotoxic lesions, chromosomal aberrations and epigenetic alterations [2,3]. Asbestos fibers may also interfere with chromosome segregation and mitosis [4].

Although asbestos has been banned in many Western countries, it is still used in several parts of the world, and some developing countries are actually increasing the industrial use of asbestos, as well as its production and importation [5,6,7]. In Western Europe, over 5,000 people with MPM die each year [8,9,10,11]. Considering the long median latency period between initial asbestos exposure and MPM diagnosis [12,13], MPM incidence is expected to peak around 2020 in Western countries [9,14,15].

Only 5%-17% of individuals heavily exposed to asbestos develop MPM [8], suggesting a genetic component in the etiology of the disease, which is also supported by reports of familial clustering [8,16,17,18] and candidate-gene association studies [8,11]. Dominant mutations in the BAP1 (BRCA1-associated protein 1) gene were recently reported to cause a new, rare cancer-prone syndrome that renders the individual susceptible to mesothelioma and melanoma, among others [19].

The aim of this study was to identify genetic risk factors that might contribute to the development of MPM. To this end, we performed a GWAS in an Italian study sample of 407 MPM cases and 389 healthy controls, and a replication study in an Australian study sample of 428 MPM cases and 1269 controls.

Table 1. Summary statistics of all the subjects included in the Italian GWAS.

| CASES | CONTROLS | CASES | CONTROLS | CASES | CONTROLS | CASES | CONTROLS |
|-------|----------|-------|----------|-------|----------|-------|----------|
| CASALE M. | TURIN | GENOA | ALL SAMPLE |
| N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) |
| Eligible | 241 (48.88) | 252 (51.12) | 91 (61.9) | 56 (38.1) | 75 (48.08) | 81 (51.92) | 407 (51.13) | 389 (48.87) |
| After QC filtering | 230 (49.25) | 237 (50.75) | 89 (68.1) | 55 (31.9) | 73 (49.32) | 75 (50.68) | 392 (51.65) | 367 (48.35) |
| GENDER | | | | | | | | |
| Males | 155 (67.39) | 162 (68.35) | 62 (69.66) | 38 (69.09) | 67 (91.78) | 56 (74.67) | 284 (72.45) | 256 (69.75) |
| Females | 75 (32.61) | 75 (31.65) | 27 (30.34) | 17 (30.91) | 6 (8.22) | 19 (25.33) | 108 (27.55) | 111 (30.25) |
| BIRTH PLACE | | | | | | | | |
| North Italy | 204 (90.27) | 186 (78.81) | 62 (69.66) | 35 (63.64) | 54 (78.26) | 53 (76.81) | 320 (83.33) | 274 (76.11) |
| Center Italy | 6 (2.65) | 12 (5.08) | 5 (5.62) | 1 (1.82) | 8 (11.59) | 5 (7.25) | 19 (4.95) | 18 (5) |
| South Italy | 14 (6.19) | 33 (13.98) | 16 (17.98) | 17 (30.91) | 4 (5.8) | 4 (5.8) | 34 (8.85) | 54 (15) |
| Sardinia | 0 (0) | 2 (0.85) | 3 (3.37) | 2 (3.64) | 1 (1.45) | 3 (4.35) | 4 (1.04) | 7 (1.94) |
| Other Caucasians | 2 (0.88) | 3 (1.27) | 3 (3.7) | 0 (0) | 2 (2.9) | 4 (5.8) | 7 (1.82) | 7 (1.94) |
| ASBESTOS EXPOSURE | | | | | | | | |
| Non exposed | 4 (2.06) | 54 (22.78) | 3 (3.37) | 18 (32.73) | 3 (3.37) | 0 (0) | 2 (2.9) | 4 (5.8) |
| Medium exposed | 106 (54.64) | 103 (43.46) | 33 (37.08) | 25 (45.45) | 7 (9.59) | 22 (39.33) | 146 (41.01) | 150 (40.87) |
| High exposed | 84 (43.3) | 80 (33.76) | 53 (59.55) | 12 (21.82) | 56 (76.71) | 12 (16) | 193 (54.21) | 104 (28.34) |
| Age (mean±s.e.) | 66.46±10.81 | 66.42±12.26 | 68.53±9.28 | 68.70±7.69 | 64.16±13.70 | 63.44±14.47 | 66.5±11.01 | 66.12±12.06 |

doi:10.1371/journal.pone.0061253.t001

Results

The general characteristics of the Italian study sample, after quality controls (QC), are reported in Table 1 (392 MPM cases and 367 controls; 540 males, 219 females). A total of 339,879 SNPs were included in the analyses. The principal component analysis (PCA) (Figure S1) showed population stratification with two distinct clusters, which was further confirmed by K-mean analysis (data not shown). After correction of the regression analyses by PCA-cluster, the inflation factor was ≈1.03 for both the overall and the exposed-only samples (Quantile-Quanitle, QQ plots, Figure S2). Manhattan plots of the two-sided logistic regression analyses (per allele additive model) are also reported (Figure 1).

The genotyped SNPs with the highest significance levels are listed in Table 2. The imputed SNPs with the highest significance levels are listed in Table S1. Nine intragenic SNPs (7 genotyped and 2 imputed) were located in genes. When analyzing these nine genes in a Gene Set Enrichment Analysis (GSEA, File S1), significant enrichment involving MMP14 and ADAMTS2 was shown for gene-ontology (GO, File S1) biological processes including lung development (P=0.0087), respiratory tube development (P=0.0087), respiratory system development (P=0.0087), metalloendopeptidase activity (P=0.0140), and metallopeptidase activity (P=0.0210) (Table S2).

When the GSEA (File S1) was extended to SNPs with a significance level of P≤10⁻³ in the regression analysis (additive model, 201 genes), another metalloendopeptidase, namely MMP8, was included in the gene list, further reinforcing the putative role of the metalloendopeptidase pathway in MPM.

Haplotype association was investigated in the Italian study sample for the 20 genes/chromosomal regions with the highest significance levels. The most significant haplotype associations were found in the chromosomal region 3p24.2, where the THRBB
gene is located \((P = 2.04 \times 10^{-7})\), and in 19q13.42 \((P = 7.02 \times 10^{-7})\) (Table S3), strengthening the importance of these chromosomal regions.

Seven chromosomal regions were significantly associated with MPM in the region-based analysis \((P < 0.0025, \text{Table 3, Figure 2, Figure S3}) [20]\). The gene-based analysis confirmed the significance of the \(THRB\) gene \((P = 2.29 \times 10^{-5})\) and showed a borderline significance for the \(PVT1\) gene \((P = 0.02)\) (Table 3). Finally, the regional GO (File S1) process-based analysis supported the involvement of the metalloendopeptidase and metallopeptidase GO (File S1) processes (Table 3, \(P = 0.0005\) and \(0.0039\), respectively).

We detected a substantial improvement in accuracy comparing the first multivariate model, which used asbestos exposure as a predictor and adjusted for demographic covariates, with the second one, which also included 10 selected SNPs with independent effects (Table 4). The average Akaike Information Criterion (AIC) and area under ROC curve (AUC) across 10,000 random splits of the entire Italian study sample were 871.34 and 0.76 for the first model, and 730.27 and 0.86 for the second model, respectively (Figure 3, Table 4). The analysis stratified by center (Casale Monferrato versus Turin-Genoa) confirmed the stability of the risk estimates and 95% CIs (data not shown).

The first multivariate model confirmed asbestos exposure as the main risk factor for MPM [high exposure: OR 17.33, 95% CI 9.28–32.37, \(P = 2.6 \times 10^{-16}\); low exposure: OR 8.01, 95% CI 4.41–14.54, \(P = 8.52 \times 10^{-12}\) (Table 4). The second model, which included the genetic component, showed that the 10 selected SNPs had an independent contribution to MPM risk (Table 4), and also increased the estimate for the effect of asbestos exposure (high

---

**Figure 1. Manhattan plot of genotyped SNPs from logistic additive model. A) all samples, B) exposed samples.**

doi:10.1371/journal.pone.0061253.g001
## Table 2. Italian top 12 genotyped SNP list (2-tailed logistic regression, n = 759 overall, n = 593 exposed only).

| CHR Location | SNP     | Ref. Allele | OR (95% CI) | P      | Typed  | Gene Name | Left Gene | Right Gene | Group |
|---------------|---------|-------------|-------------|--------|--------|-----------|-----------|------------|-------|
| 6q21          | rs742109| A           | 0.55(0.43–0.71) | 2.70×10⁻⁶ | Genotyped | PRDM1     | ATG5      |            | OVERALL |
| 3q26.2        | rs7632718| A           | 1.83(1.42–2.37) | 3.71×10⁻⁶ | Genotyped | SLC7A14, CLDN11 | CLDN11 | RPL22L1 | EXPOSED |
| 3p24.2        | rs9833191| C           | 0.54(0.41–0.71) | 7.67×10⁻⁶ | Genotyped | THRB      | NR1D2     | MIR4792   | EXPOSED |
| 5q23.1        | rs1508805| A           | 1.85(1.41–2.44) | 1.04×10⁻⁵ | Genotyped | PRR16     | FTMT      |            | EXPOSED |
| 1q25.2        | rs2501618| A           | 2.18(1.53–3.10) | 1.49×10⁻⁵ | Genotyped | CEP350    | TOR1AIP1  | QSOX1     | EXPOSED |
| 5q35.3        | rs4701085| G           | 1.84(1.39–2.44) | 1.93×10⁻⁵ | Genotyped | ADAMT52   | ZNF354C   | AX747985  | EXPOSED |
| 4q22.1        | rs4290865| A           | 1.98(1.44–2.71) | 2.16×10⁻⁵ | Genotyped | FAM190A   | GRID2     |            | EXPOSED |
| 13q14.3       | rs9536579| A           | 0.54(0.40–0.72) | 7.67×10⁻⁶ | Genotyped | OLFMS     | MIR1297   |            | OVERALL |
| 7p21.2        | rs3801094| A           | 1.75(1.35–2.27) | 2.52×10⁻⁵ | Genotyped | ETV1      | AR14A     | DGX8      | OVERALL |
| 8q24.21       | rs7841347| A           | 0.60(0.47–0.76) | 2.60×10⁻⁵ | Genotyped | PVT1      | MYC       | TMEM75    | OVERALL |
| 15q21.1       | rs10519201| A           | 2.36(1.57–3.56) | 3.82×10⁻⁵ | Genotyped | SHC1      | EID1      | SECSBP2L  | EXPOSED |
| 22q12.3       | rs5756444| G           | 0.60(0.47–0.76) | 3.95×10⁻⁵ | Genotyped | CSF2RB2   | C22orf33/TEX33 |            | EXPOSED |

## Table 3. Region-, Gene- and GO process-based analysis on top SNPs (1-tailed binomial test, n = 759, alpha 0.0025, alpha = 0.01, alpha = 0.025, respectively).

| Region/Gene/GO processes based | Cytogenetic Band | Position (from - to) | Number of SNPs | Significant SNPs | P   |
|--------------------------------|------------------|----------------------|----------------|-----------------|-----|
| -                              | 1q25.2           | (178792161–178267165) | 5              | 4               | 8.31×10⁻⁴ |
| -                              | 3p24.2           | (24311166–24397755)   | 17             | 7               | 3.86×10⁻⁴ |
| -                              | 3q26.2           | (171668688–171738200) | 12             | 6               | 9.47×10⁻⁵ |
| -                              | 4q22.1           | (92842088–92925574)   | 11             | 3               | 0.05 |
| -                              | 4q32.1           | (160680345–160763147) | 11             | 3               | 0.04 |
| -                              | 5q23.1           | (120950796–121034917) | 11             | 3               | 0.08 |
| -                              | 5q35.2           | (173515657–173599925) | 16             | 4               | 7.23×10⁻³ |
| -                              | 5q35.3           | (178859043–178654962) | 19             | 5               | 0.01 |
| -                              | 6q21             | (106565091–106738553) | 18             | 5               | 8.00×10⁻³ |
| -                              | 7p12.2           | (13877273–13974190)   | 20             | 6               | 4.36×10⁻³ |
| -                              | 7p22.2           | (4339181–4436371)     | 17             | 9               | 5.96×10⁻⁵ |
| -                              | 8q24.21          | (128837336–128935399) | 7              | 6               | 1.04×10⁻⁴ |
| -                              | 9p24.1           | (5363441–5453968)     | 12             | 5               | 0.02 |
| -                              | 12q23.3          | (107375486–107461372) | 13             | 7               | 5.78×10⁻⁵ |
| -                              | 13q14.3          | (53429288–53513774)   | 12             | 4               | 0.02 |
| -                              | 14q11.2          | (22334110–22425388)   | 13             | 2               | 0.14 |
| -                              | 15q14            | (34831353–34470568)   | 13             | 5               | 2.04×10⁻³ |
| -                              | 15q21.1          | (46959609–47047893)   | 18             | 2               | 0.23 |
| -                              | 19q13.42         | (59189856–59266559)   | 9              | 1               | 0.47 |
| -                              | 22q12.3          | (3560028–35754794)    | 19             | 5               | 0.03 |
| CEP350                      | 1q25.2           | (179933906–180093734) | 17             | 2               | 0.31 |
| THRB                        | 3p24.2           | (24162088–24541322)   | 54             | 15              | 2.29×10⁻³ |
| SLC7A14                     | 3q26.2           | (170167538–171715102) | 13             | 2               | 0.16 |
| SDK1                        | 7p22.2           | (3341374–4303003)     | 90             | 5               | 0.61 |
| PVT1                        | 8q24.21          | (128808953–129119976) | 34             | 7               | 0.02 |
| METALLOENDOPEPTIDASE        | -                | -                     | 197            | 19              | 4.65×10⁻³ |
| METALLOPEPTIDASE            | -                | -                     | 470            | 32              | 0.04 |

Plosone | www.plosone.org | April 2013 | Volume 8 | Issue 4 | e61253
exposure: OR 45.28, 95% CI 21.52–95.28, \( P < 2 \times 10^{-16} \); low exposure: OR 15.31, 95% CI 7.78–30.14, \( P = 2 \times 10^{-15} \).

SNP validation and replication

The Italian and Australian study samples showed a marked degree of heterogeneity (I² statistics, range 0.62–0.97) [21] (Table S5). None of the 12 genotyped SNPs with the highest significance levels in the Italian study were found in the Australian replication study (Table S4), and nor of these were confirmed by the meta-analysis (Table S5). Nevertheless, when a regional analysis was performed in the Australian study sample, we found significant associations in five chromosomal regions (3q26.2, 4q32.1, 7p22.2, 14q11.2, 15q14) that have reported to be altered in mesothelioma (Table 5) [20].

Gene expression analysis in blood and in normal pleural tissue

Gene expression analysis on lymphocytes from Italian healthy subjects (Text S1) showed a possible expression Quantitative Trait Locus (eQTL) for the \( \text{PVT1} \) (rs7841347) gene (non-parametric Kruskal-Wallis test \( P < 0.001 \)) (Figure 4). However, expression analysis from Italian healthy subjects pleural tissue stratified by \( \text{PVT1} \) rs7841347 genotypes did not show any gradient, although a statistically significant difference \( (P = 0.01) \) was found (Figure S4). Published expression data [22] (Text S1) confirmed the dysregulation of \( \text{MMP14}, \text{THRB} \) and \( \text{MYC} \) genes in MPM, supporting our results.

SNP predictive functional analysis

Using the GenomePipe tool, none of the SNPs with the highest significance levels included in the present analysis might predict damage, nor were they located in a regulatory or splicing site.
Even when SNPs in Linkage Disequilibrium (LD) with our top SNPs (LD $r^2 > 0.8$ as measured by pairwise $r^2$) were included in the analysis no evidence of functional properties of the proxy SNPs was found. LD refers to two different populations, i.e. HapMap TSI from Tuscany (Italy) and CEU (HapMap3, File S1), for a total of 33 and 72 SNPs respectively.

**Discussion**

In order to identify genetic risk factors that might contribute to the development of MPM, we performed a GWAS on 407 Italian MPM cases and 389 controls.

We performed an independent replication study in an Australian sample, which included 428 MPM cases (Genetic Understanding of Asbestos-Related Disease, GUARD, study) and 1,269 controls (Busselton Health Study, BHS).

Among the top SNPs identified in our Italian study sample, there were several genes previously reported to be involved in MPM or other cancer types, as well as chromosomal regions reported to be altered in MPM [20].

Although no single SNP replicated in the Australian sample, probably due to the high genetic heterogeneity between the two studies, regional analyses showed significant signals in 5 of the chromosomal regions where the Italian top SNPs are located. The chromosomal region 7p22.2 found in the replication study includes the $SDK1$ [23] and $FOXK1$ [24] genes. Interestingly, $FOXK1$ has been reported to interact with $BAP1$ [25], which was recently found to be mutated in mesothelioma [19]. Chromosomal region 7p22 is located in a fragile sequence (FRA7B) containing two miRNA genes (mir589 and mir339) and three large genes ($SDK1$, $THSD7A$, $MAD1L1$), and is highly prone to gaps and breaks in several cancers [23].

Another Italian genotyped top-signal (rs7632718) is located in the $SLC7A14$ (solute carrier family 7 member 14) gene, which lies on 3q26.2, which was one of the replicating regions in the Australian study. Although no link with MPM has been previously reported for $SLC7A14$, a chromosomal gain has been described in this region [20], suggesting a possible involvement of other genes in MPM.

The $PVT1$ (Pvt1 oncogene (non-protein coding)) gene is involved in several types of cancer [26,27,28,29,30]. It is located in a large (>300 kb) locus downstream of $MYC$ (53 Kb apart) on chromosomal region 8q24. The $PVT1$ locus produces a wide variety of spliced non-coding RNAs as well as a cluster of six annotated miRNAs: miR-1204, miR-1205, miR-1206, miR-1207-5p, miR-1207-3p, and miR-1208 [31,32]. $PVT1$ was proposed to regulate $c$-Myc gene transcription over a long distance [33].

**Table 4. Nested multivariate logistic regression models: 1) model 1, without genetic component; 2) model 2, with genetic component.**

|                  | OR 95L | OR 95U | P      | OR 95L | OR 95U | P      | GENETIC MODEL |
|------------------|--------|--------|--------|--------|--------|--------|---------------|
| LOW vs NO EXPOSURE | 8.01   | 4.41   | 14.54  | 8.52 x 10^{-12} | 15.31 | 7.78 | 30.14 | 2.86 x 10^{-15} |
| HIGH vs NO EXPOSURE | 17.33  | 9.28   | 32.37  | <2 x 10^{-16} | 45.28 | 21.52 | 95.28 | <2 x 10^{-16} |
| CLUSTER 2 vs 1    | 1.76   | 1.1    | 2.79   | 1.74 x 10^{-52} | 2.21 | 1.29 | 3.79 | 4.09 x 10^{-03} |
| rs2501618         | -      | -      | -      | 2.23   | 1.47   | 3.37   | 1.52 x 10^{-04} | dominant |
| rs9833191         | -      | -      | -      | 0.55   | 0.41   | 0.73   | 4.39 x 10^{-05} | additive |
| rs7632718         | -      | -      | -      | 1.85   | 1.41   | 2.42   | 9.07 x 10^{-06} | additive |
| rs4701085         | -      | -      | -      | 2.05   | 1.41   | 2.97   | 1.75 x 10^{-04} | dominant |
| rs73034881        | -      | -      | -      | 0.44   | 0.29   | 0.67   | 1.12 x 10^{-04} | additive |
| rs3801094         | -      | -      | -      | 1.86   | 1.39   | 2.48   | 2.78 x 10^{-05} | dominant |
| rs7841347         | -      | -      | -      | 0.51   | 0.39   | 0.67   | 1.56 x 10^{-06} | additive |
| rs10815216        | -      | -      | -      | 0.41   | 0.27   | 0.60   | 8.53 x 10^{-06} | dominant |
| rs2236304         | -      | -      | -      | 1.72   | 1.19   | 2.51   | 4.39 x 10^{-03} | dominant |
| rs7178364         | -      | -      | -      | 0.45   | 0.28   | 0.71   | 5.66 x 10^{-04} | dominant |

*adjusted for age, gender and center of recruitment.

MODEL 1: AIC = 871.3, AUC = 0.76.

MODEL 2: AIC = 730.27, AUC = 0.86.

doi:10.1371/journal.pone.0061253.t004

Figure 3. Receiver Operating Curves (ROC) for the two multivariate models including asbestos exposure 1) without and 2) with the 10 most robust and significant genetic variants.

doi:10.1371/journal.pone.0061253.g003
cancer [34]. In vitro, the rs378854-G allele has been associated with reduced binding of the transcription factor YY1, a putative tumor suppressor, and with repressed global transcription in prostate cancer [33]. The regulation of this chromosomal region is very complex, as is suggested by the association of several SNPs with different cancer types [35], and involves miRNA, lincRNA and other epigenetic regulations [36].

The gene-expression analysis on lymphocytes from Italian healthy subjects showed a possible eQTL for \textit{PVT1}. Functional studies are needed to clarify the link between \textit{PVT1}-associated SNPs, gene expression regulation and cancer risk taking into account that in our study \textit{PVT1} seems to act only at an early stage of carcinogenesis as its deregulation has not been observed at later stages in tumor tissue [22].

Two other genes that have been reported to be dysregulated in MPM, are \textit{THRB} and \textit{MMP14} [22,37]. \textit{THRB} encodes for thyroid hormone receptor beta (TR\(\beta\)), which could function as a tumor suppressor. Cell-based studies and xenograft models have demonstrated that TR\(\beta\) is a suppressor of ras-mediated cell proliferation, transformation, and tumorigenesis [38]. Moreover, TR\(\beta\) disrupts mitogenic growth factors by suppressing the activation of extracellular signal-regulated kinases and phosphatidylinositol 3-kinase signaling pathways to suppress tumor cell invasiveness and metastasis [39,40]. \textit{THRB} is located about 28 Mb telomeric to the \textit{BAP1} gene, which is mutated in MPM [19]. A down-regulation of

\begin{table}
\centering
\caption{Regional replication of Italian top signals in the Australian study for 5 out of the 20 regions.}
\begin{tabular}{lcccc}
\hline
Cytogenetic Band & BP\_start & BP\_end & p Binomial test & p Binomial test & Meta-analysis \\
\hline
3q26.2 & 171668688 & 171738200 & 9.47338E-05 & 0.01643691 & 1.61 \times 10^{-5} \\
4q32.1 & 160680345 & 160763147 & 0.042137914 & 0.000649 & 3.15 \times 10^{-4} \\
7p22.2 & 4339181 & 4436371 & 5.95584E-05 & 0.01403811 & 1.26 \times 10^{-5} \\
14q11.2 & 2234110 & 22425588 & 0.139471486 & 0.00100497 & 1.38 \times 10^{-3} \\
15q14 & 34381353 & 34470568 & 0.002040183 & 0.01305659 & 3.07 \times 10^{-4} \\
\hline
\end{tabular}
\begin{flushleft}
(1-tailed binomial test and meta-analysis).
\(^{a}\)NCBI36/hg18.
\(^{b}\)Italian study.
\(^{c}\)Australian study.
doi:10.1371/journal.pone.0061253.t005
\end{flushleft}
\end{table}

Figure 4. eQTL: \textit{PVT1} and \textit{MYC} gene-expression levels in blood cells across rs78941347 genotypes.
doi:10.1371/journal.pone.0061253.g004
THRBB has been documented in MPM versus parietal pleura [41] and it is frequently methylated/deleted in non-squamous-cell lung cancer [42].

MMP14 (matrix metallopeptidase 14) has been reported to influence overall survival in MPM cases [37], and was significantly highlighted in our enrichment analysis, together with ADAMTS2, because of their metalloendopeptidase and metallopeptidase activities. The matrix metalloproteinases are a family of zinc-containing enzymes with proteolytic activity against a wide range of extracellular proteins. Extracellular matrix proteases are involved in several steps of cancer development and progression, including angiogenesis and metastasis.

Some of the SNPs with highest significance levels were located in the genes: CEP350, ETV1 and SHCA. Although they have not been directly associated with MPM, their involvement in several cancer types has been described [43,44,45], suggesting the necessity to further investigate their possible role in MPM pathogenesis. Considering the closest flanking genes of intergenic SNPs, the following are noteworthy and could contribute to the carcinogenic process, as has been reported for other cancer types: PRDM1 [46], ATG5 [47], MYC [48], EID [49], RNL1 [50], CD274 [51].

Although our sample size is clearly a limitation for a GWAS, the Italian and the Australian study samples are, to the best of our knowledge, the largest MPM series with available DNA, as mesothelioma is a very rare cancer. A further limitation of GWAS is that they do not take into account rare variants. The availability of methods for complete genome sequencing (and the decrease of sequencing costs) will allow to circumvent the problem linked to the identification of rare variants, whose involvement should be better investigated in future studies.

The negative replication of the Italian top SNPs in the Australian study should be revised on the basis of the following considerations: i) the two studies had a marked degree of heterogeneity as shown by the I2 statistics; ii) no exposure assessment was available for the Australian control group. Notwithstanding these discrepancies, we observed an intriguing significant regional replication in the Australian study for 5 out of 20 Italian top signals.

Most of the top-signals we identified were located in chromosomal regions reported to harbor aberrant alterations in mesothelioma, and cause an at most 2-3 fold increase in MPM risk.

Moreover, asbestos exposure in our study was associated with a remarkable increase in MPM risk, which became even more evident when the contribution of genetic factors was taken into account, with a significant improvement of asbestos exposure risk estimation.

In conclusion, our results support the complementary role of genetic background in asbestos-related carcinogenesis of the pleura, indicating that genetic risk factors should be taken into account to understand MPM physiopathology, and to better define the MPM risk profile of people with a high exposure to asbestos.

Methods

Ethics statement

All MPM cases reported on in the present report gave written informed consent. This study was performed according to the principles of the Declaration of Helsinki and in agreement with ethical requirements. Approval was obtained from the Istituto Nazionale per la Ricerca sul Cancro Ethics Committee for the studies in Genoa and La Spezia, and from the Human Genetics Foundation (HuGeF) Ethics Committee for the studies in Casale Monferrato and Turin. The Australian replication study was specifically approved by the Human Research Ethics Committee of the University of Western Australia.

Italian study sample

The Italian study sample is composed of MPM cases and controls from cities located in Northern Italy: Casale Monferrato and Turin in the Piedmont Region, and Genoa and La Spezia in the Liguria Region (Table 1; details in Text S1). The study in Casale Monferrato was a population-based MPM case-control study [52], and included 241 MPM patients and 252 population controls of Italian nationality and Caucasian ethnicity. The study in Turin was a hospital-based MPM case-control study [11], and consisted of 91 MPM patients and 56 controls of Italian nationality and Caucasian ethnicity. The hospital-based study in Genoa and La Spezia included 75 incident MPM cases [53]. Controls are 81 healthy subjects or patients hospitalized for non-neoplastic/non-respiratory conditions.

All the three of the above-mentioned Italian studies were registry-based and therefore no selection criteria were applied to MPM cases; they needed only to be residing in the study area at the time of diagnosis. Only cases with a pathological diagnosis (based on histology or cytology with confirmatory immunohisto-chemical staining) were eligible for inclusion in the present analysis. Study periods in the Italian studies were different (Casale Monferrato: January 2001 to December 2006; Turin: January 2004 to October 2008; Genoa and La Spezia: April 1996 to February 2006 for cases and February 1997 and November 2006 for controls). For practical reasons, the study in Turin was limited to cases admitted to the main metropolitan hospitals.

Asbestos exposure was carefully assessed in all the Italian cases and controls. After reviewing individual occupational histories, asbestos exposure was reclassified for the overall sample by the same expert (D.M.) as “no/unlikely” (no acknowledged occupational or environmental exposure), “low” (low exposure probability, or definite low exposure), and “high” (definite and high exposure; asbestos-cement and asbestos-textile workers, insulators, shipyard workers and dockers).

Australian replication study

Australian MPM cases were part of the GUARD study, which consisted of individuals who had been exposed to asbestos and diagnosed with MPM (n = 428) and who attended a hospital clinic in Perth, Western Australia between 1988 and 2010 [54]. DNA samples and clinical data from these individuals were obtained and MPM diagnosis was confirmed after pathological, radiological and clinical review with confirmation from respective cancer registries in Western Australia (Western Australia Mesothelioma Registry) and Queensland.

The GUARD study subjects are primarily male (88.8%) with an average age of 67±10.3 years. Most BHS study subjects are female (57.4%) and the average age is 34±17.2 years. Control samples (n = 1,269), with no information on asbestos exposure, were obtained from the population-based BHS [53]. MPM cases were excluded after genotyping if they were: related to another individual, had a low call GWAS rate (<97%), were not Caucasian/European based on principal component analysis, had ambiguous sex, or had low heterozygosity compared to the rest of the sample.

SNP genotyping

Whole-genome genotyping was done on a HumanCNV370-Quad BeadChip (Illumina Inc., San Diego, CA, USA) for 716 samples. The remaining 80 samples were tested on a Human610-
Quad (which includes 100% of the HumanCNV370 BeadChip SNPs) as the HumanCNV370-Quad had been discontinued. Genotypes were assessed by GenomeStudio V2011.1 (Illumina Inc., San Diego, CA). The 12 most significant SNPs from the Italian study were individually genotyped in the Australian replication study with a 5'-nuclease assay (AppliedBiosystems, CA, USA).

Statistical analysis

Genotyping quality controls. A cut-off a genotyping call rate of 0.98 was set, leading to the exclusion of 18 study subjects. Sibling By Descent (IBD) estimation using the Identity By State (IBS) distance was used to check genotypic identity or relatedness among subjects (PLINK software [56], File S1). Subjects with IBD > 0.05 (n = 16) were considered consanguineous and excluded from further analyses. We additionally excluded three samples with an X chromosome inbreeding homozygosity estimate of about 0.5. Thirty-seven subjects (4.64%) were removed from the analysis, leaving 759 subjects (392 cases and 367 controls).

SNPs with minor allele frequency <1% (n = 15,252), those having >0.05 missing genotypes (n = 11,535) and those deviating from Hardy-Weinberg equilibrium (HWE) in the control population (P < 0.001, n = 1,157) were excluded from the analysis, for a final study data-set of 330,879 SNPs, which were analyzed for their potential association with mesothelioma.

Population structure and association analysis. The population structure was investigated by PCA (PLINK Software, File S1, Covariance Method [57]). A new discrete covariate was defined by the two principal components (Figure S1), and was included in the following logistic regression analysis. PCA results were further confirmed by the K-means clustering analysis [58] (data not shown). The effective removal of any population structure bias was checked by the λ-inflation factor parameter [59] (Figure S2).

We tested for 330,879 SNPs for their association with mesothelioma by 2-sided logistic regression analysis on a per-allele additive model after adjusting for age, gender, PCA cluster, center of recruitment and exposure level, both in the overall Italian sample (n = 759) and among exposed-only Italian subjects (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure).

We tested for 330,879 SNPs for their association with mesothelioma by 2-sided logistic regression analysis on a per-allele additive model after adjusting for age, gender, PCA cluster, center of recruitment and exposure level, both in the overall Italian sample (n = 759) and among exposed-only Italian subjects (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure).

Meta-analysis and replication. A meta-analysis of the Italian-study top 12 genotyped SNPs was done on data from the whole genome genotyping (Human610-Quad BeadChip, Illumina) of 428 cases and 1269 Australian controls of European descent (GWAMA software, File S1 [63]). A random-effects model was used as measures of the fit and the prediction power of the two models.

Prediction of functional SNPs has been carried out with several software, including GenomerPipe software, which is freely available at website of the National Institute of Environmental Health Sciences (http://www.ncbi.nlm.nih.gov/snp/GWAS.htm) and the Pupasuite3.1 software (http://pupasuite.bioinfo.cipf.es/).

Gene-expression analysis. The expression levels of the nine genes corresponding to the most common intragenic SNPs (Table 2) and of MTC, which is neighbor to PVT1, were examined using data from the HapMap (File S1) CEU gene-expression database, and the GenoPheno database [66], an internal database which includes genotypic, phenotypic, and gene-expression data from the peripheral blood of 120 healthy Italian volunteers (Text S1). We considered the average expression levels of probes and, when feasible, tested for differential expression among the three genotypes (Kruskal-Wallis test).

In addition, the mRNA levels of the PVT1, MTC and THRB genes were measured by quantitative real-time PCR in 79 normal pleural tissues from donors that underwent thoracoscopy for conditions other than MPM, who signed an informed consent form (Text S1).

A GWAS on Malignant Pleural Mesothelioma

To further explore the potential association of CEP350, SDK1, and PVT1 with mesothelioma, we performed a two-sided logistic regression analysis on a per-allele additive model after adjusting for age, gender, PCA cluster, center of recruitment and exposure level, both in the overall Italian sample (n = 759) and among exposed-only Italian subjects (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure).

We tested for 330,879 SNPs for their association with mesothelioma by 2-sided logistic regression analysis on a per-allele additive model after adjusting for age, gender, PCA cluster, center of recruitment and exposure level, both in the overall Italian sample (n = 759) and among exposed-only Italian subjects (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure).

We tested for 330,879 SNPs for their association with mesothelioma by 2-sided logistic regression analysis on a per-allele additive model after adjusting for age, gender, PCA cluster, center of recruitment and exposure level, both in the overall Italian sample (n = 759) and among exposed-only Italian subjects (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure). After Bonferroni correction, we considered alpha = 1.51 (n = 593) (high and low exposure).
Supporting Information

Figure S1 Principal Component Analysis (PCA) plots: first vs second PC. A) Cases and controls are plotted for the overall study and for each of the three study samples (Turin, Casale Monferrato and Genoa); B) birth places (Northern, Central, Southern Italy, Sardinians and Other Caucasians) are plotted for the overall study and for each of the three study samples.

TIFF

Figure S2 Supplementary figure 1: Q-Q plots for GWAS of mesothelioma in the Italian population. This Q-Q plots are based on logistic regression allelic \( P \) after standard quality control. The estimated \( \lambda \) inflation factor was <1.03. Plot A shows the Q-Q plot for the overall Italian population, whereas Plot B refers to the exposed-only population.

TIFF

Figure S3 Regional association plots for additional 4 regions (a. 3q26.2, b. 4q32.1, c. 7p21.2, d. 15q14) replicating in the Australian study. Each SNP is plotted with respect to its chromosomal location (\( x \) axis) and its log\( _{10} \) transformed \( P \) value (\( y \) axis on the left) for associations with MPM. The tall blue spikes indicate the recombination rate (\( y \) axis on the right) at that region of the chromosome. The red-outlined diamond indicate the index SNP and other diamond indicate the genotyped SNPs, the squares of the chromosome. The red-outlined diamond indicate the index CEU population. LD values were calculated only on our control population.

TIFF

Figure S4 RT-PCR of \( PVT1 \) and \( MYC \) genes-expression levels in 79 normal pleural tissue expression levels across rs78941347 genotypes.

TIFF

Table S1 Italian top 8 imputed SNP list.

DOCX

Table S2 Gene Set Enrichment Analysis.

DOCX

References

1. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2011) A review of human carcinogens: Metals, arsenic, dusts, and fibers. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Twelfth Edition 2011 2012 ed. Lyon: WHO, IARC.

2. Moshman BT, Lipmann M, Hesterberg TW, Kelsey KT, Barchowsky A, et al. (2011) Pulmonary endpoints (lung carcinomas and asbestosis) following inhalation exposure to asbestos. Journal of toxicology and environmental health Part B, Critical reviews 14: 76–121.

3. Achilli A, Oliveri A, Pala M, Metspalu E, Fornarino S, et al. (2007) Mitochondrial DNA variation of modern Tuscans supports the Near Eastern origin of Etruscans. American Journal of Human Genetics 80: 759–768.

4. Robinson BWS, Lake RA (2005) Advances in malignant mesotheloma. New England Journal of Medicine 353: 1591–1603.

5. Azari MR, Naserniaoudehli A, Mosvalhdi M, Mehrabi Y, Hatami H, et al. (2010) Risk assessment of lung cancer and asbestosis in workers exposed to asbestos fibers in brake shoe factory in Iran. Ind Health 48: 36–42.

6. Belose JE, Cox NJ, Fukagawa NK, Hirvonen A, Testa JR (2011) Factors That Impact Susceptibility to Fiber-Induced Health Effects. Journal of Toxicology and Environmental Health-Part B-Critical Reviews 14: 246–266.

7. Birns FJ (2009) Asbestos—a legacy and a persistent problem. J R Nav Med Serv 95: 4–11.

8. Neri M, Ugolini D, Dianzani I, Gemignani F, Landi S, et al. (2008) Genetic susceptibility to malignant pleural mesothelioma and other asbestos-associated diseases. Mutation Research - Reviews in Mutation Research 659: 126–136.

9. Petro J, Decarli A, La Vecchia C, Levi F, Negri E (1999) The European mesothelioma epidemic. British Journal of Cancer 79: 666–672.

10. Petro J, Hodgson JT, Matthews FE, Jones JR (1995) Continuing increase in mesothelioma mortality in Britain. Lancet 345: 535–539.

11. Betti M, Ferrante D, Padoan M, Guarerra S, Giordano M, et al. (2011) XRCC1 and ERCC1 variants modify malignant mesothelioma risk: A case-control study. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 708: 11–20.

12. Montanaro F, Rosato R, Gangemi M, Roberti S, Ricceri F, et al. (2009) Survival of pleural malignant mesothelioma in Italy: A population-based study. International Journal of Cancer 124: 201–207.

13. Martinaccio A, Binazzi A, Cauzillo G, Carone D, Zotti RD, et al. (2007) Analysis of latency time and its determinants in asbestos related malignant mesothelioma cases of the Italian register. European Journal of Cancer 43: 2722–2728.

14. Ismail-Khan R, Robinson LA, Williams CC Jr, Garrett CR, Bepler G, et al. (2006) Malignant pleural mesothelioma: a comprehensive review. Cancer Control 13: 255–263.

15. Peluchin C, Malvezzi M, La Vecchia C, Levi F, Decarli A, et al. (2004) The Mesothelioma epidemic in Western Europe: An update. British Journal of Cancer 90: 1022–1024.

16. Ascoli V, Carone D, Merler E, Barbieri PG, Romeo L, et al. (2007) Mesothelioma in blood related subjects: Report of 11 clusters among 1954 Italy cases and review of the literature. American Journal of Industrial Medicine 50: 357–369.

17. Ugolini D, Neri M, Ceppi M, Cesario A, Dianzani I, et al. (2008) Genetic susceptibility to malignant mesothelioma and exposure to asbestos: The influence of the familial factor. Mutation Research - Reviews in Mutation Research 658: 162–171.

18. de Kleer N, Alfonso H, Olen N, Reid A, Sleith J, et al. (2012) Familial aggregation of malignant mesothelioma in former workers and residents of Wittenoom, Western Australia. International Journal of Cancer (In press).

19. Testa JR, Cremo M, Prii J, Belose JE, Tan Y, et al. (2011) Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet 43: 1022–1025.
41. Roe OD, Anderssen E, Helge E, Pettersen CH, Olsen KS, et al. (2009) Genome-wide profile of pleural mesothelioma versus parietal and visceral pleura: the emerging gene portrait of the mesothelioma phenotype. PloS one 4: e6554.

20. Gray SG, Fennell DA, Mutti L, O’Byrne KJ (2009) In arrayed trays: array technology in the study of mesothelioma. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer 4: 411–425.

19. Huppi K, Volfovsky N, Runfola T, Jones TL, Mackiewicz M, et al. (2008) The C8FW, but not MYC in MYC-containing double minutes in myeloid malignancies. Cancer genetics and cytogenetics 177: 37–42.

18. Melano O, Cristaudo A, Melissari E, Di Russo M, Bonotti A, et al. (2011) A review of transcriptome studies combined with data mining reveals novel markers of malignant pleural mesothelioma. Mutation research.

17. Bosco N, Pelliccia F, Rocchi A (2010) Characterization of FRAT7, a human common fragile site mapped at the 7p chromosome terminal region. Cancer genetics and cytogenetics 202: 47–52.

16. Beck-Engeser GB, Lum AM, Huppi K, Caplen NJ, Wang BB, et al. (2008) Pvt1-encoding microRNA in tumor suppressor genes contributing to metastasis in a mouse model of thyroid follicular carcinoma. Mol Cell Biol 28: 100–108.

15. Carramusa L, Contino F, Ferro A, Minafra L, Perconti G, et al. (2007) The matrix metalloproteinase 14 (MMP-14) as potential tumour target. PLoS One 4: 7514–7523.

14. Storlazzi CT, Fioretos T, Paulsson K, Strombeck B, Lassen C, et al. (2004) Involvement of MRE11A and XPA gene polymorphisms in the modulation of asbestos-related factors in asbestos-related malignancies. Polymorphisms in DNA repair genes as risk factors for asbestos-related malignant mesothelioma in a general population study. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 599: 124–134.

13. Ugolini D, Neri M, Canessa PA, Casilli C, Catrambone G, et al. (2008) The CREST biopoieses: a tool for molecular epidemiology and translational studies on malignant mesothelioma, lung cancer, and other respiratory tract diseases. Cancer Epidemiology, biomarkers & prevention : a publication of the International Association for the Study of Lung Cancer 17: 3013–3019.

12. Ricceri F, Porcedda P, Allione A, Turinetto V, Polidoro S, et al. (2011) GWAMA: software for genome-wide association studies. Nature Genetics 38: 904–909.

11. Marchini J, Howie B, Myers S, McVean G, Donnelly P (2007) A new multipoint imputation method for the next generation of genome-wide association studies. Nature Genetics 38: 904–909.

10. De Klein NH, Armstrong BK, Musk AW, Hobs MA (1989) Cancer mortality in relation to measures of occupational exposure to crocidolite at Wittenoom Gorge in Western Australia. British journal of industrial medicine 46: 529–536.

9. Creaney J, Olsen NH, Bruns F, Dick M, Musk AW, et al. (2010) Serum mesothelin for early detection of asbestos-induced cancer malignant mesothelioma. Cancer Epidemiol Biomarkers Prev 19: 2238–2246.

8. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, et al. (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics 38: 904–909.

7. Hartigan JA, Wong MA (1979) Algorithm AS 136: A K-Means Clustering Algorithm. Journal of the Royal Statistical Society Series C (Applied Statistics) 28: 100–108.

6. Devlin B, Roeder K (1999) Genomic control for association studies. Biometrics 55: 997–1004.

5. Development Core Team R (2009) A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available: http://www.R-project.org, 2009.

4. Marchini J, Howie B, Myers S, McVean G, Donnelly P (2007) A new multipoint imputation method for genome-wide association studies by imputation of genotypes. Nat Genet 39: 501–506.

3. de Klein NH, Armstrong BK, Musk AW, Hobs MA (1989) Cancer mortality in relation to measures of occupational exposure to crocidolite at Wittenoom Gorge in Western Australia. British journal of industrial medicine 46: 529–536.

2. Howie BN, Donnelly P, Marchini J (2009) A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 5: e1000259.

1. Magi R, Morris AP (2010) GWAMA: software for genome-wide association meta-analysis. BMC Bioinformatics 11: 288.

PLOS ONE | www.plosone.org 11 April 2013 | Volume 8 | Issue 4 | e61253