

Project Final Report

Title of the project : Molecular changes and genetic susceptibility in relation to air pollution and environmental tobacco smoke: a case-control study in non-smokers nested in the epic investigation		
Acronym of the project : GEN - AIR		
Type of contract : Shared cost		Total project cost (in euro) 1.147,115 €
Contract number QLK4-CT-1999-00927	Duration 48 months	EU contribution (in euro) 1.147,115 €
Commencement date April, 1 st . 2000		Period covered by the final report April 1 st , 2000 - April 1 st , 2004
<u>PROJECT COORDINATOR</u>		
Name : Paolo Vineis	Title: Professor	Address Universita' di Torino via Santena 7 101026 Torino
Telephone : + 39 11 670.65.25	Telefax: +39 11 670.66.92	E-mail address: paolo.vineis@unito. it
Key words gene-environment interactions , air pollution , passive smoking , cancer , molecular epidemiology		
World wide web address http://www.isi.it/18/9/yes/ssnsss/14/project_detail.html		

List of participants :

- Paolo Vineis, Department of Biomedical Sciences and Human Oncology, University of Torino, and ISI Foundation, Torino, Italy (Coordinator)
- Gerard Hoek, University of Utrecht, The Netherlands
- Elio Riboli, Christian Malaveille, Pierre Hainaut, International Agency for Research on Cancer, 150, Cours Albert Thomas, 69372 Lyon, France (Contractor)
- Michal Krzyzanowski, WHO AIR QUALITY European Centre for Environment and Health, Bonn Office, Goerre Strasse 15, 53113 Bonn, Germany (Contractor)
- Marco Peluso, C.S.P.O. Centro per lo Studio e la Prevenzione Oncologica , Firenze , Italy (Contractor)
- Luisa Airoidi, Istituto per le Ricerche Farmacologiche Mario Negri, via Eritrea 62 20157 Milano Italy (Contractor)
- Herman Autrup University of Aarhus - Department of Occupational and Environmental Medicine Vennelyst Boulevard 6, bld 260 - 800 Aarhus C Denmark (Contractor)
- Seymour Garte - Genetics Research Institute Onlus Genetics Research Institute - Viale Lazio 26 20135 Milano , Italy (Contractor)
- Alison Dunning, Dept. of Oncology, University of Cambridge, Box 193 Addenbrooke's Hospital Hills Road CB2 2QQ Cambridge UK (Contractor)
- Giuseppe Matullo, Department of Genetics, Biology and Biochemistry, University of Turin, Via Santena 19, 20126 Torino Italy (Contractor)
- Antonello Provenzale, Maurizio Manuguerra, I.S.I. Foundation - Institute for Scientific Interchange, viale Settimio Severo 65, 10133 Torino Italy (Contractor)

Acknowledgements

The following persons have actively collaborated to the success of GENAIR:

For technical and administrative coordination:

Sabrina Bertinetti, University of Torino and ISI Foundation

For exposure assessment:

Federica Vigna-Taglianti, University of Torino

Joelle Colosio and Helene Desqueyroux, ADEME, France

Mario Mansi, Ildikò Tamàs and Franco Santonastasi, Napoli, Italy

Daniele Grechi, ARPAT - Dipartimento di Firenze, Italy

Allesandro Borgini, Varese, Italy

Mauro Maria Grosa, ARPA Torino, Italy

Armando Tuero, Consejeria de Medio Ambiente, Oviedo, Spain

Lluís Cirera Suárez, Consejería de Sanidad y Consumo, Cartagena, Spain

Iñaki Bañares, Dirección de Medio Ambiente (D.F.G.), Donostia, Spain

Zuazo Onagoitia, Pedro, Medio Ambiente, Pamplona Spain

Juan Contreras González, Consejería de Medio Ambiente, Granada, Spain

Ole Hertel and Ole Raaschou-Nielsen, Copenhagen, Denmark

Jeff Broughton, AEA Technology Environment

Manfred Lotz, Ulrich Berger and Hannelore Schlegel, LUA Brandenubrug, Germany

Hr. Siegel and Helmut Scheu-Hachtel, UMEG, Germany

Bertil Forsberg and Lars Modig, University Umea, Sweden

Henric Nilsson, city of Malmo, Sweden

Patrick Steiberger, RIVM, the Netherlands

For laboratory analyses:

Armelle Munnia, CSPO Firenze

Simonetta Guarrera, ISI Foundation

Silvia Polidoro, ISI Foundation

Sara Gamberini, ISI Foundation

Federica Saletta, ISI Foundation

Benedetta Mannari, CSPO Firenze

Chiara Grissini, CSPO Firenze

Chiara Varano, CSPO Firenze

For statistical analyses:

Fabrizio Veglia, ISI Foundation

Carlotta Sacerdote, University of Torino

Andrew Rundle, Columbia University, New York

SUMMARY

The overall aim of the project was to **quantitatively assess the effects of air pollution and Environmental Tobacco Smoke (ETS)** on cancers of the lung, bladder, pharynx, and larynx in **non-smokers** in nine European countries (**UK, Sweden, Denmark, Germany, The Netherlands, France, Spain, Italy and Greece**). This was done with a nested case-control design in the EPIC investigation (500,000 volunteers), by the means of biomarkers of exposure and susceptibility. Exposure assessment was made by experts on the basis of the already available questionnaires plus objective information on air pollution in European cities. In addition, we measured cotinine in the blood for ETS exposure assessment, and we assessed the effect of dose and length of exposure to environmental pollutants on biological endpoints, such as DNA and hemoglobin adducts. We studied the **dose-response relationship** between adducts and disease according to genotypes for metabolism of the relevant carcinogens and DNA repair. We also evaluated the relationships between cancer and mutations in relevant genes (p53, ras), as measured in the blood DNA according to a newly developed technique. A final goal was to evaluate the protective effect of fruit and vegetable consumption on the same cancers, in interaction with environmental exposures.

The main results of the study can be summarized as follows:

- We have identified 4051 subjects (1074 cases and 2977 controls) who meet the protocol criteria. Of these subjects, 2410 had blood samples (846 cases and 1564 controls); DNA samples were available for 1645 subjects
- Exposure to air pollution was estimated at individual level on the basis of the address and information from monitoring stations and about traffic. The following pollutants were assessed: CO, NO₂, PM₁₀, TSP, O₃ and SO₂. There was considerable variation in pollution levels among centers, but also considerable inter-individual variation as expressed by the standard deviation. For most pollutants there was a decrease in the concentrations between the first time period of assessment (1990-94) and the second (1995-99). This was particularly evident in the case of SO₂, but it was not so for O₃ (which, on the opposite, showed a slight increase in France and the Netherlands). As expected, the variability between centers was larger than between countries. Also changes between the two time periods were more evident for single centers. A limit of the study was the availability of only one address for the subjects, at recruitment.
- Individual exposure data were available for 197 cases and 556 matched controls. We found an odds ratio of 1.14 (95% CI 0.78-1.67) for each increment of 10 ug/m³ in exposure to NO₂. However, no association was found among never smokers, while the OR for ex-smokers since at least 10 years was 2.22 (1.01-4.87). After adjustment by duration of smoking the OR in ex-smokers was 2.69 (1.01-7.20), and after adjustment by education, BMI, physical activity, intake of fruit, vegetables, meat and energy the OR was 3.69 (1.24-10.94). We also found an association with residence nearby heavy traffic roads, particularly after adjustment by cotinine and in ex-smokers. Therefore, we have found that higher exposure to (traffic-related) air pollutants can increase the risk of lung cancer in ex-smokers since at least 10 years, perhaps because air pollutants cause clonal expansion of mutated cells. No association was found in never smokers, possibly for lack of power. Advantages of our study are the prospective design, and thorough control for potential confounders (including cotinine measurement in serum). Our observation is highly relevant from a Public Health perspective, since the European population includes many millions of ex-smokers.
- Lung cancer, upper respiratory cancer, and respiratory deaths, all among ex-smokers and never smokers, were studied in relation to information on

Environmental Tobacco Smoke present in questionnaires. We have found that both the whole group of respiratory conditions, and lung cancer alone, were associated in a statistically significant manner to self-reported ETS exposure at the time of recruitment. The association was consistently observed after stratification by country. Odds ratios were: in former smokers 2.21 (95% confidence interval 0.6-7.9) according to the case-control analysis, and 2.98 (1.0-8.7) according to the full cohort analysis; for never smokers they were respectively 1.73 (0.7-3.8) and 1.15 (0.7-2.0). It is unlikely that the stronger association in ex-smokers is explained by confounding by smoking habits in the past. One possible explanation is that ex-smokers are more susceptible to low level exposure to ETS because they already have mutations in their cells. ETS exposure during childhood showed an association with lung cancer, particularly in never smokers; the association was statistically significant for daily exposure for many hours. The biological plausibility of a causal association between ETS exposure and lung cancer is reinforced by the observation that having 3 or more polymorphic genes increases the odds ratio to 3.96, with a dose-response relationship with the number of at risk polymorphisms. Cotinine levels were not clearly and consistently associated with the risk of lung cancer. This could be expected, since previous authors have stressed the limitations of cotinine as a biomarker of exposure. Cotinine is an expression of the last 24 hours of exposure, and is valuable mainly to exclude current smoking rather than estimating long-term exposure to ETS.

- DNA adducts seemed to be associated with the subsequent risk of lung cancer. When we considered the trend from undetectable to levels below median and above median, the trend was statistically significant only among never smokers. Odds ratios were: in never smokers 2.07 (95% CI 1.02-4.19; p-value for trend 0.04), and in ex-smokers 0.85 (0.50-1.44; p-value 0.54). A non-statistically-significant association in never smokers was also shown for upper aero-digestive cancers (mouth, pharynx, larynx), that share with lung cancer some risk factors (in particular exposure to polycyclic aromatic hydrocarbons). We computed correlation coefficients between log(adducts) and the air pollutants we have considered. A positive association was found for O₃, and a statistically significant negative association was found for PM₁₀. However, when all the pollutants were included in a multivariate model, only the association with O₃ persisted.
- We have studied plasmatic DNA and mutations in p53 and ras genes. The amount of DNA was highly variable across EPIC centres (p<0.0001). In multivariate analysis, the amount of plasma DNA, as a continuous variable, was apparently associated with overall cancer onset, and also with death from COPD, after adjustment for age and centre. The only single type of tumour showing a statistically significant association with the amount of DNA was leukemia. Inclusion of smoking status did not affect estimates. When the analyses were stratified by time since recruitment (below or above 36 months), the increased OR were limited to the more recent period of recruitment, i.e. a time elapsed between blood drawing and disease onset lower than 36 months. A strong association with COPD below 36 months is particularly noteworthy (OR=12.7); also the association with leukemia is stronger (OR=2.37), and statistically significant, below 36 months.

- A total of 374 specimens were analysed for *TP53* mutations (143 cases and 231 controls). Nine mutations were detected, including 6 in subjects who developed a cancer during follow-up (crude OR: 3.3, 95% CI: 0.8 - 13.4). The mutations were distributed in exons 5 to 9, which contain the vast majority of the somatic mutations described in most cancers. The median time between detection of *TP53* mutation in the plasma and diagnosis of cancer was 13.3 months (range: 1.8-32.2 months). No association was found with any of the putative risk factors or protective factors (environmental tobacco smoke, air pollution levels, dietary habits), but numbers were too small for a reliable inference. Of the 374 subjects who were tested for both *TP53* and *KRAS*, 34 developed a bladder cancer during the follow-up. Remarkably, 5 of these 34 subjects (14.7%) were positive for either *TP53* (3 subjects) or *KRAS* (2 subjects). Thus, presence of a mutation in plasma DNA was predictive for development of bladder cancer (crude OR: 7.79; 95% CI: 2.1 - 28.6; OR (for matched pairs 11.69, 95% CI 1.25-109), but not of other types of cancers included in the study.
- Our analysis of smoking data in the EPIC study, based on the TSCE mathematical model, suggests that smoking affects mainly the net proliferation rate of cells. With this model the tumour distribution by age was accurately predicted. However, these conclusions are still provisional because several competing mathematical models and their inherent assumptions are under scrutiny. The same models will be applied to data on air pollution and ETS.