



AIRGENE

Air Pollution and Inflammatory Response in Myocardial Infarction Survivors: Gene-Environment Interaction in a High Risk Group

Executive Summary



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EXECUTIVE SUMMARY

BACKGROUND

Ambient air pollution has been associated with an increased risk of hospital admission and mortality in potentially susceptible subpopulations, including myocardial infarction (MI) survivors.

OBJECTIVES

The aim of the study was to assess the role of cardiovascular disease risk factors, including ambient air pollution and polymorphisms in candidate genes, in determining the inter- and the intra-individual variability in plasma IL-6, CRP and fibrinogen concentrations in MI survivors. The study was designed to assess specifically the role of candidate gene polymorphisms hypothesized to lead to a modification of the short-term effects of ambient air pollution.

METHODS

A multicenter epidemiological study was conducted between May 2003 and July 2004 in six European cities: Helsinki, Stockholm, Augsburg, Rome, Barcelona, and Athens. Outcomes of interest are plasma concentrations of the pro-inflammatory cytokine interleukin 6 (IL-6) and the acute phase proteins C-reactive protein (CRP) and fibrinogen. In total, 1,003 MI survivors were recruited and assessed with at least 2 repeated clinic visits without any signs of infections up to three days prior to the blood withdrawal. The 5,813 blood samples collected correspond to an average of 5.8 repeated clinic visits per subject (97% of the scheduled 6 repeated visits per person). At baseline, participants' behavior and medical histories were determined by interview, and blood pressure, height, weight, cholesterol levels, and N-Terminal proB-type natriuretic peptide (NT-proBNP) were measured. Short-term exposures/behaviors and medication intake were recorded at each visit. DNA from each individual was collected and genotyped. We investigated 97 tagging single nucleotide polymorphisms (SNPs) in thirteen candidate genes, all involved in the innate inflammatory pathway, among them *IL6*, *fibrinogen* (*FGA*, *FGB*, and *FGG*) and *CRP*. Hourly data on particle number concentrations (PNC), mass concentrations of diameter less than 10 μm (PM_{10}) and 2.5 μm ($\text{PM}_{2.5}$), gaseous pollutants and meteorological data were collected at central monitoring sites in each city. Additive mixed models using P-Splines, where the spline coefficients are estimated in the mixed model framework, were chosen for the analyses. We implemented this approach in a SAS macro which can be used in a wide variety of contexts. For the air pollution analyses, city specific confounder models were built for each blood marker separately adjusting for meteorology, time-varying and time-invariant confounders. Effect estimates for the air pollutants were computed for each city separately and subsequently pooled using meta-analysis methodology.

RESULTS

Particulate air pollution was highest in the three Southern European countries, with Rome being the most polluted city, and Helsinki being the lowest. We analyzed the short-term association between air pollutants and CRP, IL-6 and fibrinogen, controlling for weather and a range of other potentially important confounders. Pooled results show a clear increase in IL-6 when concentrations of PNC and NO₂ were elevated 12-17 hours before the clinic visit. Cumulative exposure (5-day-average) to PM_{2.5} and PM₁₀ was associated with increased fibrinogen concentrations. No consistent associations were found for CRP. Results indicate an immediate response to ambient air pollution on the IL-6 level, which might lead to the production of acute phase proteins, as seen in increased fibrinogen levels, whereas for CRP no associations were detectable, possibly due to a widespread intake of lipid-lowering medication in the study population.

Promoter polymorphisms within *FGA* and *FGB* were associated with modifications of the associations between 5-day moving averages ambient particulate matter and plasma fibrinogen levels. More than threefold higher effect estimates were observed for subjects with the homozygote minor allele genotype of *FGB* rs1800790 as compared to the overall estimated association between PM₁₀ and fibrinogen. The difference between the estimates for PM₁₀ for subjects with the homozygote minor allele genotype compared to subjects with the homozygote major allele was more than eightfold. The effect estimates were twofold higher for the homozygote major allele genotype of *FGA* rs2070011. This non-statistically significant effect modification was observed although higher fibrinogen concentrations were observed for the minor allele. Effect estimates for PM_{2.5} were generally slightly smaller and interactions less pronounced than for PM₁₀. Associations varied between cities but these variations were within the range of the expected statistical variability. Analyses considering different induction times suggested that the gene-particulate matter associations were consistent for cumulative exposures to ambient particulate matter.

We performed separate analyses of the association between air temperature and CRP, IL-6 and fibrinogen. Meteorological data were obtained from the country-specific network stations. The association was analyzed using an additive model with random patient effects. The effect estimates are presented as percent changes of the geometric mean for CRP and IL-6 levels and as percent changes of the arithmetic mean for fibrinogen based on a 10°C increase of air temperature. A decrease of the mean air temperature of one day and four days before the blood withdrawal as well as of the 5-day-average was associated with a 3 to 4 percent increase in the geometric mean of CRP-level. The city-specific effect estimates were all negative except for one city. Although they ranged between -14.3% and +6.6%, no evidence for heterogeneity was observed. Sensitivity analyses with different confounder models showed that the effect estimates were very robust. Correspondingly, a significant increase of IL-6 of 3 percent was observed for a decrease in the 5-day-average air temperature whereas fibrinogen showed an increase of around 1 percent associated with a decrease of air temperature with a lag of 3 days. The results suggest that a decrease in air temperature, particularly the average temperature of the last 5 days, leads to an increase in CRP- as well as IL-6-level whereas fibrinogen seems to react to temperature changes after 3 days. In susceptible patients this might lead to an additional risk for cardiovascular events and provides a hint for the observed seasonal variation in death from ischemic heart disease and stroke in the elderly.

Among survivors of a MI, IL-6 levels are associated with many of the traditional cardiovascular risk factors. Age, time of day, body mass index (BMI), pack-years of

smoking, NT-proBNP, systolic blood pressure, HDL cholesterol, persistent cough/phlegm, and statins use were significantly and independently associated with IL-6 after adjustment for city, recurrent MI, baseline alcohol intake, current active smoking, tea consumption, and extreme anger or stress. Gender was not independently associated with IL-6. Patients with elevated NT-proBNP, respiratory symptoms, or obesity had higher IL-6 concentrations and may potentially be at greater risk for coronary artery disease progression. In four IL-6 SNPs in high linkage disequilibrium (LD) with each other, the heterozygote and rare homozygote were associated with an increased mean plasma IL-6 level. For another SNP an inverse direction of effect was suggested (higher mean IL-6 level for the common homozygote). For each SNP, an allelic dose-response relationship was suggested. Analysis of variability showed that while the rare homozygotes of the 4 correlated SNPs were associated with higher mean levels of IL-6, they demonstrated less variability within individuals but higher variability between individuals compared to heterozygotes and common homozygotes.

We identified SNPs and haplotypes from the fibrinogen genes cluster (*FGA*, *FGB* and *FGG*) related with the levels of fibrinogen and we assessed these SNPs' association in relation with IL-6 and CRP levels. We also examined whether SNPs and haplotypes could modify the physiological intra-individual variability of fibrinogen levels. The analyses of fibrinogen were limited to five cities (Augsburg, Barcelona, Helsinki, Rome, Stockholm), as the quality of the plasma samples for fibrinogen in Athens did not meet the required high standards. 21 SNPs located in the 3 fibrinogen genes were analyzed; haplotypes were reconstructed over the three genes. Additive mixed models were used to assess the association between the SNP and the fibrinogen levels. Eight SNPs of *FGA* and *FGB* were associated with fibrinogen levels in the overall population, while the SNPs' association with the fibrinogen levels were not consistent in all 5 cities. Two different haplotypes in *FGA* and 3 in *FGB* were associated with fibrinogen levels. The levels of IL-6 had a significant impact on the associations between SNPs/haplotypes and fibrinogen levels. One SNP and 1 haplotype in *FGA*, and 1 SNP and 1 haplotype in *FGB* were implicated in the intra-individual variability of fibrinogen levels during the follow-up period. The present study suggests a crucial role of the *FGA* and *FGB* SNPs and haplotypes in plasma fibrinogen concentration and intra-individual variability in patients with history of MI. We have also identified for the first time, SNPs/haplotypes on *FGA* and *FGB* playing a possible role in the IL-6 - fibrinogen interaction.

We found two SNPs within the *CRP* gene, rs1800947 and rs1205, of which the minor alleles were strongly associated with lower baseline levels of CRP ($p < 2 \times 10^{-7}$). The haplotype tagged by those two SNPs was associated with the greatest reduction in plasma levels of CRP ($p = 4 \times 10^{-7}$). Additionally, we found the minor allele of a rare variant (rs1800894) in the promoter region of the interleukin-10 (*IL10*) gene to be significantly associated with greater individual variability of CRP level ($p = 4 \times 10^{-4}$). The present study suggests that inter-individual variation of the CRP concentration could be regulated by the *CRP* gene itself, while the intra-individual variability seems to be influenced by factors acting upstream in the inflammatory cascade.

Dietary habits were evaluated through a semi-quantitative Food Frequency Questionnaire (FFQ), while adherence to a traditional Mediterranean diet was assessed by a protective diet score that incorporated important characteristics of this diet. An inverse relationship was found between protective diet score and logCRP ($b = -0.029$, $p = 0.021$) and logIL-6 ($b = -0.019$, $p = 0.01$) levels, after adjusting for center, age, sex, BMI, physical activity and smoking status. Moreover, moderate red wine intake (1-12 wine glasses per month) was

associated with lower levels of CRP and IL-6 ($p < 0.05$). Adherence to the traditional Mediterranean diet was associated with a reduction of the concentrations of inflammatory markers in MI survivors from different ethnic groups. This may, in part, explain the beneficial actions of this diet on various chronic diseases such as cardiovascular diseases and cancer.

CONCLUSIONS

Ambient particles were associated with increases in IL-6 and fibrinogen concentrations. No association was found for ambient air pollution and CRP, which suggests that current treatment of MI survivors with lipid-lowering medication, in particular statins may protect them from the effect of ambient particles. The data presented here suggests that genetically determined susceptibility to ambient particulate matter may be due to polymorphisms which alter early physiological responses such as transcription of fibrinogen. As fibrinogen is a substrate for coagulation and an acute phase protein, subjects with these frequent SNPs may have increased risks not only due to constitutionally higher fibrinogen concentrations, but also due to an augmented response to environmental inflammatory stimuli such as ambient particulate matter. Cold temperatures were associated with increases in CRP indicating that these effects may be mediated through different pathways. Classical risk factors such as high BMI, age, gender, high cholesterol levels were associated with elevated inflammatory markers. Genetic variation in the inflammatory marker genes also determined their levels. When comparing the size of air pollution effects to the effects of other constant factors, between 2-fold to 10-fold larger effects were determined for the time-invariant person characteristics. Overall, inflammation in MI survivors is determined by a number of time-invariant as well as time-varying environmental factors, which increases the risk for subsequent events in this high-risk group of the population.

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