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DNA methylation and exposure to ambient air pollution in two prospective cohorts

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ABSTRACT

Long-term exposure to air pollution has been associated with several adverse health effects including cardiovascular, respiratory diseases and cancers. However, underlying molecular alterations remain to be further investigated. The aim of this study is to investigate the effects of long-term exposure to air pollutants on (a) average DNA methylation at functional regions and, (b) individual differentially methylated CpG sites. An assumption is that omic measurements, including the methylome, are more sensitive to low doses than hard health outcomes.

This study included blood-derived DNA methylation (Illumina-HM450 methylation) for 454 Italian and 159 Dutch participants from the European Prospective Investigation into Cancer and Nutrition (EPIC). Long-term air pollution exposure levels, including NO_2, NO_x, $PM_{2.5}$, PM_{coarse}, PM_{10}, $PM_{2.5\ absorbance}$ (soot) were estimated using models developed within the ESCAPE project, and back-extrapolated to the time of sampling when possible. We meta-analysed the associations between the air pollutants and global DNA methylation, methylation in

Abbreviations: CpG, 5′–C–phosphate–G–3′, a cytosine and guanine separated by one phosphate; CPMA, cross-phenotype meta-analysis; ELISA, enzyme-linked immunosorbent assay; ESCAPE, European Study of Cohorts for Air Pollution Effects; EPIC-Italy, The Italian part of the European Prospective Investigation into Cancer and Nutrition; EPIC-NL, The Dutch part of the European Prospective Investigation into Cancer and Nutrition; EpWAS, epigenome-wide association study; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography–mass spectrometry; LINE1, long interspersed nuclear elements; LUMA, luminometric methylation assay; NO_2, nitrogen dioxide; NO_x, nitrogen oxide; PM, particulate matter; $PM_{2.5}$, particulate matter with a diameter smaller than 2.5 μm; PM_{10}, particulate matter with a diameter smaller than 10 μm; PM_{coarse}, particulate matter with a diameter smaller than 10 μm and bigger than 2.5 μm; $PM_{2.5abs}$, absorbance of the $PM_{2.5}$ filter, a measure for soot

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functional regions and epigenome-wide methylation. CpG sites found differentially methylated with air pollution were further investigated for functional interpretation in an independent population (EnviroGenoMarkers project), where (N = 613) participants had both methylation and gene expression data available.

Exposure to NO_2 was associated with a significant global somatic hypomethylation (p-value = 0.014). Hypomethylation of CpG island's shores and shelves and gene bodies was significantly associated with higher exposures to NO_2 and NO_x. Meta-analysing the epigenome-wide findings of the 2 cohorts did not show genome-wide significant associations at single CpG site level. However, several significant CpG were found if the analyses were separated by countries. By regressing gene expression levels against methylation levels of the exposure-related CpG sites, we identified several significant CpG-transcript pairs and highlighted 5 enriched pathways for NO_2 and 9 for NO_x mainly related to the immune system and its regulation.

Our findings support results on global hypomethylation associated with air pollution, and suggest that the shores and shelves of CpG islands and gene bodies are mostly affected by higher exposure to NO_2 and NO_x. Functional differences in the immune system were suggested by transcriptome analyses.

1. Introduction

Ambient air pollution includes gaseous components such as nitrogen oxides, benzene, and sulfur dioxide as well as particulate matter. The latter consists of acids, organic chemicals, metals and soil or dust particles of varying aerodynamic diameters. Because of their small size, these particles can be inhaled deeply into the lungs and deposited in the alveoli. The smallest particles can penetrate the lung epithelium and reach the blood stream (Brook et al., 2010). Exposure to air pollution is associated with adverse health outcomes including cardiovascular and respiratory diseases (Brook et al., 2010; Nawrot et al., 2011). There is increasing evidence that toxic substances in ambient air at levels that are at or below the current limits in the European Union may increase the risk of lung cancer. Analyses of large European cohorts showed that a 10 $\mu g/m^3$ increase in PM_{10} was associated with a 22% (95% confidence interval [CI]: 3 to 45%) greater risk for lung cancer, while a 5 $\mu g/m^3$ increase in $PM_{2.5}$ was associated with an 18% (95% CI: − 4 to 46%) increased risk (Raaschou-Nielsen et al., 2013). However, the potential modes of action of air pollutants are not well understood. Also, hard health outcomes such as cancer or cardiovascular diseases may not be sensitive enough (or require very large cohorts) to demonstrate low-dose effects.

Previous research suggests that inflammation, oxidative damage, and mitochondrial dysfunction (Demetriou et al., 2012; Mostafavi et al., 2015; Pettit et al., 2012) may be the underlying mechanisms leading from exposure to air pollution to health outcomes. Global DNA hypomethylation induces genomic instability, for instance through chromatin structure modelling (You and Jones, 2012), loss of imprinting, and increased activation of oncogenes (Feinberg and Tycko, 2004). Epigenetic studies report an inverse association between global methylation and long-term exposure to ambient air pollution (Baccarelli et al., 2009; De Prins et al., 2013; Janssen et al., 2013; Sanchez-Guerra et al., 2015; Tao et al., 2014), especially affecting 5-hydroxymethylcytosine methylation (Sanchez-Guerra et al., 2015). However, analyses of global methylation in these studies were based on the investigation of LINE-1 or Alu regions, or by means of HPLC, ELISA, LUMA or LC-MS, and analyses of functional regions in the genome have not yet been performed in relation to long-term exposure to air pollution.

Methylation levels at specific loci in genes including tissue factor F3, interferon gamma (*IFN-γ*), interleukin 6 (*IL-6*), toll-like receptor 2 (*TLR-2*), intercellular adhesion molecule 1 (*ICAM-1*), ten-eleven translocation (*TET1*) have been reported to be affected by exposure to air pollution (Bind et al., 2015; Bind et al., 2014; Lepeule et al., 2014; Somineni et al., 2015). Recent studies using Illumina Infinium Human Methylation 450 K technology (a) identified CpG sites whose methylation levels were affected by short and mid-term exposure to particulate matter (Panni et al., 2016); (b) investigated the association between long-term ambient air pollution exposure and DNA methylation (candidate sites and global) in monocytes of adults (Chi et al., 2016); (c) identified differential offspring DNA methylation in mitochondria-related genes in association with NO_2 exposure during pregnancy (Gruzieva et al., 2016). Also, long-term exposure to air pollution has been found associated with epigenetic aging measures (Ward-Caviness et al., 2016).

Using data from the Italian and Dutch components of the European Prospective Investigation into Cancer and Nutrition cohort study (EPIC), we investigate if differences in global DNA methylation or DNA methylation at certain functional regions could be induced by long-term exposure to air pollutants and could be used as a marker of low-dose effects. In addition, we adopt an epigenome-wide association study (EpWAS) approach to identify possible CpG sites whose methylation levels are affected by long-term exposure to air pollution. Using an independent population in which DNA methylation profiles and gene expression are available in the same participants (N = 613), we linked full-resolution gene expression levels data and methylation levels at exposure-related CpG sites to potentially characterise the functional consequences of these methylation alterations.

2. Methods

We first assessed the association between ambient air pollution estimates and global DNA methylation including functional regions in the two EPIC cohorts. In the second part of the manuscript, we performed an epigenome-wide association study. Finally, we investigated the transcripts associated with air pollution CpGs in the EnviroGenoMarkers study.

2.1. Study populations

EPIC is a multi-centre prospective cohort based on healthy, middle-aged subjects who agreed, following an active invitation, to participate in the study and to have their health status followed up for the rest of their lives. The present study included participants from two large population-based cohorts: the Italian and Dutch components of the EPIC study (EPIC-Italy, N = 47,749 and EPIC-Netherlands (EPIC-NL), N = 33,066); (Beulens et al., 2010; Palli et al., 2003).

- The Italian samples originate from two case-control studies on breast cancer (N = 231) (van Veldhoven et al., 2015) and colorectal cancer (N = 304), from two EPIC – Italy centres: Varese and Turin.
- The Dutch samples originate from a longitudinal study on weight change in healthy women nested within EPIC-NL (N = 170).

The rationale and design of the EPIC study have been described elsewhere (Riboli and Kaaks, 1997). The EPIC study protocol was approved by the ethical review boards of the International Agency for Research and Cancer (IARC) and by the local participating centres. For all participants, anthropometric measurements and lifestyle variables were collected at recruitment (1993–1998) through standardized