



Modifying Effect of a Common Polymorphism in the Interleukin-6 Promoter on the Relationship between Long-Term Exposure to Traffic-Related Particulate Matter and Heart Rate Variability

Martin Adam^{1,2*}, Medea Imboden^{1,2}, Eva Boes^{1,2,3}, Emmanuel Schaffner^{1,2}, Nino Künzli^{1,2}, Harish Chandra Phuleria^{1,2}, Florian Kronenberg³, Jean-Michel Gaspoz⁴, David Carballo⁵, Nicole Probst-Hensch^{1,2}

1 Swiss Tropical and Public Health Institute, Basel, Switzerland, **2** University of Basel, Basel, Switzerland, **3** Division of Genetic Epidemiology, Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck Medical University, Innsbruck, Austria, **4** Department of Community Medicine, Primary Care and Emergency medicine, University Hospitals, Geneva, Switzerland, **5** Cardiology, Department of Internal Medicine Specialties, University Hospitals, Geneva, Switzerland

Abstract

Background: Exposure to particulate matter (PM) has been associated with an increase in many inflammatory markers, including interleukin 6 (*IL6*). Air pollution exposure has also been suggested to induce an imbalance in the autonomic nervous system (ANS), such as a decrease in heart rate variability (HRV). In this study we aimed to investigate the modifying effect of polymorphisms in a major proinflammatory marker gene, interleukin 6 (*IL6*), on the relationship between long-term exposure to traffic-related PM₁₀ (TPM₁₀) and HRV.

Methods: For this cross-sectional study we analysed 1552 participants of the SAPALDIA cohort aged 50 years and older. Included were persons with valid genotype data, who underwent ambulatory 24-hr electrocardiogram monitoring, and reported on medical history and lifestyle. Main effects of annual average TPM₁₀ and *IL6* gene variants (rs1800795; rs2069827; rs2069840; rs10242595) on HRV indices and their interaction with average annual exposure to TPM₁₀ were tested, applying a multivariable mixed linear model.

Results: No overall association of TPM₁₀ on HRV was found. Carriers of two proinflammatory G-alleles of the functional *IL6* -174 G/C (rs1800795) polymorphism exhibited lower HRV. An inverse association between a 1 µg/m³ increment in yearly averaged TPM₁₀ and HRV was restricted to GG genotypes at this locus with a standard deviation of normal-to-normal intervals (SDNN) (GG-carriers: -1.8%; 95% confidence interval -3.5 to 0.01; p_{interaction(additive)} = 0.028); and low frequency power (LF) (GG-carriers: -5.7%; 95%CI: -10.4 to -0.8; p_{interaction(dominant)} = 0.049).

Conclusions: Our results are consistent with the hypothesis that traffic-related air pollution decreases heart rate variability through inflammatory mechanisms.

Citation: Adam M, Imboden M, Boes E, Schaffner E, Künzli N, et al. (2014) Modifying Effect of a Common Polymorphism in the Interleukin-6 Promoter on the Relationship between Long-Term Exposure to Traffic-Related Particulate Matter and Heart Rate Variability. PLoS ONE 9(8): e104978. doi:10.1371/journal.pone.0104978

Editor: Roger A. Coulombe, Utah State University, United States of America

Received: April 11, 2014; **Accepted:** July 16, 2014; **Published:** August 18, 2014

Copyright: © 2014 Adam 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 Swiss National Science Foundation (grant numbers 33CSCO-108796, 3247BO-104283, 3247BO-104284, 3247-065896, 3100-059302, 3200-052720, 3200-042532, 4026-028099), the Federal Office for Forest, Environment and Landscape, the Federal Office of Public Health, the Federal Office of Roads and Transport, the canton's government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino, Valais and Zurich, the Swiss Lung League and the canton's Lung League of Basel Stadt/ Basel Landschaft, Geneva, Ticino, Valais and Zurich. No form of payment was given to any of the authors to produce the manuscript. The study received funding through peer-reviewed grants and donations from non-profit organizations as listed above. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: martin.adam@unibas.ch

Introduction

Exposure to air pollution as well as decreased heart rate variability (HRV) as measured by specific indices (SDNN, TP, LF, HF) have been associated with increased cardiovascular morbidity and mortality in longitudinal studies, both in patients with myocardial infarction and in healthy persons [1–3]. Heart rate variability (HRV) refers to the beat-to-beat variation in heart rate

and is a non-invasive measure of the autonomic regulation of cardiac rhythm [4,5]. A reduction in different HRV indices can reflect both an increase in sympathetic or a decrease in parasympathetic tone [2]. Several studies have introduced HRV as an intermediate factor between acute exposure to air pollution and cardiovascular morbidity, showing that PM exposure is associated with increased heart rate and reductions in most indices of HRV [6,7]. A recent meta-analysis including 29 studies

supported an inverse relationship between HRV and short-term particulate air pollution [8]. Most studies, including a recent double-blind randomized cross-over study, often focused on acute exposure effects and persons with pre-existing cardiovascular diseases, diabetes mellitus or the elderly [2,9]. Studies on the chronic impact of air pollution on HRV are scarce [10,11]. The American Heart Association (AHA) recently stated that studies on long-term effects of air pollution on HRV and cardiovascular health are a major unresolved issue. In a previous study analysing the same study population for the association between long-term TPM_{10} and HRV, we found an impact of air pollution on participants treated for hypertension and heart disease [2].

Air pollution exposure is associated in the short-term with elevated levels of inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL-6), fibrinogen and platelet activation, especially in elderly and diabetic subjects, but also in healthy populations [12–15]. Long-term circulating interleukin 6 levels in prospectively collected blood are predictive of subsequent risk of coronary heart disease, making *IL6* a strong candidate gene for modifying air pollution effects on coronary heart disease related phenotypes including mortality [16–18].

To date, no study has investigated the main association of *IL6* polymorphisms on HRV and their modifying effect on the air pollution-HRV association in the general population. Investigating the modifying effect of genetic variants in interleukin 6 (*IL6*) encoding, a key marker of inflammatory response, on the association between traffic-related air pollution and heart rate variability, can improve the understanding of the mediating role of inflammatory processes. Using data from the “Swiss cohort study on air pollution and lung and heart disease in Adults” (SAPALDIA) we found novel evidence that genetic variation in the pleiotropic *IL6* gene alters heart rate variability and its association with long-term exposure to traffic-related particulate air pollution (TPM_{10}).

Methods

Ethics statement

The study complies with the declaration of Helsinki and ethical approval for the SAPALDIA study was given by the Swiss Academy of Medical Sciences, the national ethics committee for clinical research (UREK, Project Approval Number 123/00) and the Cantonal Ethics Committees for each of the eight examination areas (Ethics commissions of the cantons Aargau, Basel, Geneva, Grisons, Ticino, Valais, Vaud and Zurich). Participants were required to give written consent before any part of the health examination was conducted either globally (for all health examinations) or separately for each investigation.

Study population

This study is part of the SAPALDIA cohort which was designed to investigate the long-term health effects of air pollution [19,20]. In 1991 a random population sample of white adults aged 18–60 years was recruited from eight areas in Switzerland featuring distinct geographic and environmental conditions. While the baseline examination of 9651 persons focused on respiratory health, at follow-up in 2002 (SAPALDIA2) a random sample of in total 1846 persons aged ≥ 50 years was invited to undergo a 24-hr electrocardiogram (ECG) recording to assess HRV. Exclusion criteria were general or spinal anaesthesia within 8 days ($n = 5$), myocardial infarction within 3 months before the examination ($n = 2$), taking digitalis ($n = 6$), an artificial internal pacemaker ($n = 0$), and ECG recordings showing atrial fibrillation ($n = 12$), less than 18 hours of recording ($n = 73$) [21] or of insufficient quality

($n = 6$) [22]. From the 1742 participants with 24-hr ECG recordings, we finally included 1552 participants with valid data on cardiovascular risk factors, and TPM_{10} long-term exposure.

HRV measurements and definition

Measurement of HRV and assessment of cardiovascular risk factors in SAPALDIA have been previously described [22] (Methods S1 in File S1). In brief, participants were asked to follow their regular daily routine during the recording period. Digital devices with a frequency response of 0.05–40 Hz and a resolution of 128 samples/s, recording on three leads (V_1 , altered V_3 with the electrode on the left mid clavicular line on the lowest rib, and altered V_5 with the electrode on the left anterior axillary line on the lowest rib) were used. The mean duration of the 24-hr ECG recordings was 22.1 (SD 2.3) hours. The standard deviation of all normal RR (NN) intervals (SDNN) and the following frequency domain variables were calculated: total power (TP) (≤ 0.40 Hz), ultra-low frequency (ULF) power (≤ 0.0033 Hz), very low frequency (VLF) power (0.0033–0.04 Hz), low frequency (LF) power (0.04–0.15 Hz), high frequency (HF) power (0.15–0.40 Hz), and the ratio between LF and HF (LF/HF).

Air pollutant exposure estimation

To assess the effect of long-term exposure to traffic-related pollution on HRV, exposure was defined as the average concentration of traffic-related PM_{10} (TPM_{10}) over 10 years. Given that total PM_{10} is not specific to near-road traffic-related pollutants, we focused instead on TPM_{10} to capture the high spatial variability of those source specific pollutants (Methods S1 in File S1). The dispersion modeling approach is described elsewhere [23]. In brief, these Gaussian plume models used the traffic-specific PM_{10} on-road emissions from light- and heavy-duty vehicles, buses, and motorcycles, taking into account diurnal variability, weekday-weekend differences as well as seasonal patterns. Co-variables used in the dispersion models were wind speed and direction, temperature, mixing height, and atmospheric stability classes. By linking participants residential addresses to annual mean TPM_{10} exposure concentrations derived for 200×200 meter grid cells from dispersion models and historical trend data of central site measurements, exposure was individually assigned to all residences of the participants reported for the period between 1990 and 2000 [24]. The PM_{10} exposure modeling and details of the individual exposure assignment have been described before [23,24]. Since information on short-term TPM_{10} was not available, short-term PM_{10} exposure was used to assess short-term effects on HRV in a sensitivity analysis approach. Short-term PM_{10} was assessed using averaged fixed monitoring station pollution measurements of the same day or up to one week preceding the Holter recording. Data was included from measurement stations nearest to the subjects' home addresses. Subjects living farther than 5 km from a station or having moved within the previous year were excluded for this sensitivity analysis.

Selection of IL6 genetic variants and genotyping

The single functional nucleotide polymorphism (SNP) in the *IL6* gene locus (rs1800795; *IL6* -174 G/C) was selected as the main candidate to capture the common genetic variation in this chromosomal region. This SNP has shown to be associated with circulating IL-6 blood concentrations [25,26]. The G allele was identified as the proinflammatory allele in previous epidemiological and experimental studies. In an explorative supplementary analysis benefitting from available GWAS data, three additional *IL6*-SNPs (rs2069827, rs2069840, rs10242595) were included as their genotyping call rates were $\geq 97.5\%$ and they were haplotype

tagging SNPs or associated with cardiovascular phenotypes or in high linkage disequilibrium (LD) with such SNPs (Tables S1–S2 in File S1).

Genomic DNA was isolated from EDTA-buffered whole blood using PUREGENETM DNA Purification Kit (GENTRA Systems, Minneapolis, USA) [19]. Genotypes of three SNPs (rs2069827, rs2069840, rs10242595) were assessed using the Sequenom's MassARRAY system (Sequenom, San Diego, USA) by performing iPLEX single base primer extension and matrix-assisted laser desorption ionization time-of-flight mass spectrometry as described elsewhere [27]. Genotypes of one SNP (rs1800795) were assessed using 5'-nuclease fluorescent realtime PCR (TaqMan) genotyping assay (Applied Biosystems, Rotkreuz, Switzerland). End-point detection was done using a 7000 ABI System detection device (ABI, Rotkreuz, Switzerland) [28]. For genotyping quality-control, a random selection of >5% of the samples were genotyped twice.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was assessed, irrespective of HRV measurement, for the total genotyped SAPALDIA population of 6055 subjects by using the STATA (Table S1 in File S1) `genhwi` command for global κ -statistic testing. Haploview 4.2 was used for analysis of Lewtonin's linkage disequilibrium (LD) calculating the metrics D-prime and R-squared (Table S2 in File S1).

The SAPALDIA subpopulation for this study is consistent with our previous analyses on the association between TPM₁₀ and the log-transformed outcome variable (HRV) [10]. An effect estimate represents percent change in geometric mean. Covariates included in the analysis were chosen in accordance with previous analysis [10] and supplemented by alcohol consumption, passive smoking, diabetes and noise exposure. Covariates included in the regression model were: sex (male as reference), age (years), age², body mass index (bmi, kg/m²), bmi², smoking status (never as reference, ever), daily exposure to environmental tobacco smoke (ETS, none as reference, <3 hours, >3 hours), daily alcohol consumption (<1 drink as reference, ≥1 drink), weekly physical activity (to the point of getting out of breath or sweating) (none as reference, between 1/2 and 2 hours, >2 hours), uric acid concentration (micromol/L), hypertension (no as reference, yes), heart disease (no as reference, yes), diabetes (no as reference, yes), street and railway noise exposure (mean dB(A) per night), seasonal effects (sine and cosine functions of the day of examination with a period of 1 year). High sensitive C-reactive protein (mg/l) was additionally included in the model to guard against potential confounding by unmeasured proinflammatory short-term effects, such as acute air pollution effects. A random intercept in a mixed regression model was included to adjust for potential residual clustering of data within the eight areas of Switzerland.

The underlying genetic model of the SNP effect was defined, using additive and dominance contrasts in the regression models and applying the Cochran-Armitage test to assess potential deviation from the additive model (Methods S1 in File S1). In such a model a significant dominance contrast measure represents deviation from the additive allele effect. To further differentiate in between a dominant and a recessive effect, we considered the direction of the association of the dominance parameter. In the paper we only show p-values of the most likely inheritance mode (additive, dominant or recessive) of the tested main effects of the *IL6* polymorphisms, as well as the interactions of the *IL6* polymorphisms with TPM₁₀.

Main effects of *IL6* gene variants were derived from the mixed model described above and adjusted for long-term TPM₁₀. *IL6*

variants and interaction terms between TPM₁₀ and the *IL6* variants were entered into the regression model separately for each genotype. Genotype-specific effect estimates for TPM₁₀ were obtained by including the stratum-specific product terms of the genotype with TPM₁₀ exposure in covariate-adjusted mixed linear models.

In sensitivity analysis, to control the possible acute effects of ambient air pollution on HRV, we also adjusted the core mixed model for the community average ambient PM₁₀-level of the 3 days prior to the Holter recording. Furthermore, we replaced TPM₁₀ exposure with the annual NO₂ exposure averaged over the previous 10 years. In a previous publication we had not found a main effect of NO₂ on HRV [11].

As this is an exploratory study investigating potential inflammatory mechanisms through a genetic key marker of inflammatory response and a functional SNP in this gene, selected a priori, we did not adjust for multiple testing [29].

All tests were two-sided with a significance level of 0.05. Statistical analyses were performed using STATA, version 12 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP) and SAS V 9.2 (SAS Institute, Cary, NC, 2008).

Results

As described in Table 1, the mean age of the study population was 60.3 (SD 6.2) years with a mean BMI of 26.7 kg/m². Among the subjects, 50.4% were females, 57% ever smokers, 21.5% were exposed to second hand smoke, 46.3% consumed alcohol regularly, 41.2% were physically inactive, 47.7% reported hypertension, 9.5% diabetes and 24.7% a heart disease.

The overall analysis including the whole study population (n = 1552) showed no general association of any HRV parameter with TPM₁₀ except for the LF/HF ratio which was 3.6% lowered (95% confidence interval (95%CI), -6.9 to -0.2) [10]. The functional *IL6*-174 G/C (rs1800795; n = 1549) polymorphism, which was previously associated with circulating IL-6 [30,31], was associated with some of the HRV metrics in either an additive or a dominant manner (Table 2). Compared with -174 GG genotypes, participants having only one or no G-risk allele exhibited higher SDNN (GC: 3.8%; 0.8 to 6.8; CC: 4.0%; 95%CI, 0.1 to 8.0; $p_{\text{additive}} = 0.015$) and TP (GC: 7.5%; 95%CI, 0.7 to 14.9; CC: 7.6%; 95%CI, -1.4 to 17.5; $p_{\text{additive}} = 0.041$), while the LF/HF ratio decreased (GC: -8.8%; 95%CI, -14.7 to -2.5; CC: -1.3%; 95%CI, -9.6 to 8.0; $p_{\text{dominant}} = 0.021$). No significant overall association was observed for HF and LF power. None of the other *IL6* SNPs included in the supplementary explorative analysis were statistically significantly associated with HRV (Tables S3–S7 in File S1).

Table 3 presents the results for the interaction between TPM₁₀ exposure and the *IL6*-174 G/C on change in HRV. The *IL6*-174 G/C polymorphism showed interactions with traffic-related PM₁₀ and the following HRV metrics: SDNN ($p_{\text{interaction(additive)}} = 0.028$) and LF ($p_{\text{interaction(dominant)}} = 0.049$) (Table 2). A 1 µg/m³ increment in yearly averaged TPM₁₀ level was associated with a decrease in SDNN of 1.8% (95% confidence interval (95%CI), -3.5 to 0.01) and in LF power of 5.7% (95%CI, -10.4 to -0.8) in participants with the -174 GG genotype. No significant interaction with TPM₁₀ exposure and the other HRV metrics (TP, HF power and LF/HF ratio) was observed but for all parameters except HF, TPM₁₀ decreased heart rate variability strongest among GG genotype carriers. All other *IL6* SNPs included in the supplementary analysis showed a weaker or no

Table 1. Characteristics of the study population (N = 1552).

Characteristics	All (n = 1552)	
Gender		
Men	770	49.6%
Women	782	50.4%
Age	60.3	±6.2
Lifestyle factors		
Smoking status		
Never Smokers	668	43.0%
Ever Smokers	884	57.0%
ETS exposure		
None	1218	78.5%
<3 h/ day	217	14.0%
≥3 h/ day	117	7.5%
Alcohol		
<1glass / day	834	53.7%
≥1glass / day	718	46.3%
Physical activity		
None	640	41.2%
0.5–1.5 h/ week	508	32.7%
≥2 h/ week	404	26.0%
Cardiovascular health		
Systolic blood pressure (mmHG)	132.1	±19.1
Diastolic blood pressure (mmHG)	81.7	±10.5
BMI (kg/m ²)	26.7	±4.3
Diabetes		
No	1404	90.5%
Yes	148	9.5%
Hypertension		
No	811	52.3%
Yes	741	47.7%
Heart disease		
No	1168	75.3%
Yes	384	24.7%
Noise Exposure		
Street noise (dB(A))	38.3	±7.7
Railway noise (dB(A))	6.0	±10.7
Air pollution		
Average annual traffic related PM ₁₀ (μg/m ³) ^a	2.3	±1.3
3-days lag PM ₁₀ (μg/m ³) ^b	22.0	±14.5
IL6 polymorphisms		
candidate SNP: rs1800795 (–174G/C)	1549	
GG	584	37.7%
GC	704	45.4%
CC	261	16.8%
SNPs included in exploratory analyses: rs2069827	1524	
GG	1264	82.9%
GT	246	16.1%
TT	14	0.9%
rs2069840	1526	
CC	638	41.8%
CG	706	46.3%

Table 1. Cont.

Characteristics	All (n = 1552)	
GG	182	11.9%
rs10242595	1529	
GG	664	43.4%
GA	686	44.9%
AA	179	11.7%

ETS indicates environmental tobacco smoke exposure; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index;

^aAnnual average over 10 years previous to the study.

^bAverage over 3 days previous to the HRV measurement.

doi:10.1371/journal.pone.0104978.t001

effect modification of TPM₁₀ on HRV, respectively, and did not add additional information (Tables S8–S12 in File S1).

In order to guard against confounding by short-term exposure of PM₁₀, which was not considered in the main and interaction effect model, we included a mean daily PM₁₀ exposure measurement over the three days previous to the ECG assessment. Associations reported for long-term exposure to TPM₁₀ with *IL6* polymorphisms and their interactions differed only slightly when adjusting additionally for short-term PM₁₀ exposure (data not shown). When we replaced TPM₁₀ with NO₂ we observed interactions with *IL6*-174 G/C that were highly comparable to those observed for TPM₁₀ (data not shown).

Discussion

To our knowledge, this is the first report on both, the main effect of genetic variation in *IL6* on HRV and the interaction of *IL6* polymorphisms with air pollution on HRV. In this candidate-approach association study in the general population, we observed the previously described functional *IL6*-174G/C promoter polymorphism to be associated with HRV (SDNN; TP; LF; HF). The association of TPM₁₀ with decreased HRV parameters was restricted to carriers of the *IL6*-174 GG genotype. The results lend support to the notion that inflammatory mechanisms mediate part of the air pollution related effects on heart rate variability.

The pleiotropic cytokine IL-6 is central in acute and chronic inflammation by inducing hepatic synthesis of acute phase proteins and by modulating the inflammatory response. In other studies, circulating IL-6 as well as hs-CRP were inversely associated with parasympathetic nervous system tone measured as LF of HRV both, in healthy individuals and patients with CVD or diabetes [32,33]. Separate lines of evidence provide support to the role of genetic variation in *IL6* in low grade systemic inflammation and, albeit less consistently, cardiovascular disease risk. First, several studies investigated the association of *IL6* gene variants with circulating IL-6 concentrations and with gene activity, most of them focusing on the SNP *IL6*-174 G/C (rs1800795) [30,31]. The *IL*-174 G-allele, which was associated with lower HRV in this current study was previously associated with higher IL-6 blood concentrations, higher *IL6* gene transcriptional activity, and higher inducible IL-6 responses [34,35]. The restriction of the TPM₁₀ effect on lowering HRV is therefore consistent with an effect in a subgroup predisposed to a proinflammatory state. Second, increased serum levels of repeatedly measured IL-6 were observed among survivors of myocardial infarction who carried the G-allele of this SNP [31]. Third, *IL6* polymorphisms and in particular the -174 G/C variant were previously associated with various cardiovascular disease outcomes and risk factors, including ischemic cerebrovascular events [36], coronary heart disease

[37,38], high blood pressure [37,39], total cholesterol, LDL, fasting glucose, BMI [40], carotid artery compliance and carotid intima media thickness [39,41] as well as arterial stiffness and pulse pressure [42]. Yet, in a meta-analysis from 2006 [38] the authors concluded that most of the studies looking at the 174 G/C promoter polymorphism and its association with risk of coronary heart disease (CHD) were case-control studies showing heterogeneous associations between the genotypes and risk of cardiovascular heart disease. In contrast, we excluded all severely ill patients to specifically assess potential effects on HRV in the general population.

Evidence to support the role of systemic low grade inflammation in mediating susceptibility of the autonomic nervous system to air pollution is sparse. A small panel study reported short-term effects of particulate air pollution on decreased HRV to be stronger among elderly persons with high levels of CRP and fibrinogen [43]. While studies on the interaction between air pollution and inflammatory gene variants may be better suited for assessing susceptibility to long-term exposures, data on gene-air pollution interactions in cardiovascular health area generally very sparse as recently reviewed by Zanobetti et al. [44]. In fact, all results published on gene-air pollution interactions in relation to HRV were derived from a single study, the Normative Ageing Study of men, and focused on acute effects of air pollution. Results from this study generally support the role of pulmonary or systemic oxidative stress in linking air pollution and HRV [45,46]. The SAPADIA cohort team previously reported on the modifying effect of antioxidative *GST* gene polymorphisms (*GSTM1* and *GSTT1* gene deletions and the *GSTP1* SNP Ile105Val) in the association of HRV with the inflammatory risk factors second-hand smoke and obesity in the general population [47]. These *GST* polymorphisms did not modify the association between TPM₁₀ and HRV in this study.

A major strength of our study is the population-based design and the detailed information available on participants. The exposure assessment of TPM₁₀ with individual exposure estimates taking residential history into consideration has advantages to assess long-term exposure to traffic-related air pollution, providing good differentiation. Due to the detailed information on numerous cardiovascular risk factors, we were able to control for major confounding factors. Furthermore, we were able to guard our analysis of long-term TPM₁₀ effects against confounding by short-term PM₁₀ exposure. The likely absence of confounding by short-term air pollution is further supported by the fact that non-inclusion of high sensitive C-reactive protein as model covariate did not materially alter the results (Tables S13-S14 in File S1).

This study has also a number of limitations. First, the results presented refer to statistical associations and interactions. The low

Table 2. Unadjusted and adjusted^a geometric means of the HRV indices and percent differences in HRV indices by traffic-related PM₁₀ (TPM₁₀) and by IL6-174G/C genotypes (N = 1549).

HRV parameters	Exposures	Crude Mean, 95%CI	Adjusted Mean, 95%CI ^a	% change	lower CI	upper CI	p-value ^b	p-value ^c
SDNN	TPM ₁₀	131.6	131.6	0.08 ^d	-1.16	1.32	0.905	
	GG	127.9	128.6					0.015 (1)
	GC	133.7	133.3	3.76 ^e	0.82	6.79	0.012	
Total Power (TP)	CC	134.5	133.6	3.98 ^e	0.07	8.04	0.046	
	TPM ₁₀	3678.4	3678.4	-0.05 ^d	-2.76	2.74	0.972	
	GG	3481.7	3510.9					0.041 (1)
Low Frequency Power (LF)	GC	3791.2	3778.6	7.54 ^e	0.69	14.86	0.030	
	CC	3825.8	3788.8	7.62 ^e	-1.42	17.48	0.101	
	TPM ₁₀	220.2	220.2	-2.07 ^d	-5.70	1.71	0.279	
High Frequency Power (HF)	GG	212.9	214.9					0.296 (1)
	GC	220.4	221.4	2.68 ^e	-4.96	10.93	0.503	
	CC	236.1	228.3	5.64 ^e	-4.71	17.12	0.297	
LF/HF Ratio	TPM ₁₀	68.6	68.6	1.07 ^d	-3.64	6.00	0.663	
	GG	63.6	64.3					0.116 (1)
	GC	72.4	72.3	12.70 ^e	2.01	24.49	0.019	
LF/HF Ratio	CC	70.2	68.6	7.13 ^e	-6.19	22.35	0.309	
	TPM ₁₀	3.2	3.2	-3.60 ^d	-6.89	-0.19	0.038	
	GG	3.3	3.3					0.021 (2)
LF/HF Ratio	GC	3.0	3.1	-8.80 ^e	-14.68	-2.53	0.007	
	CC	3.4	3.3	-1.27 ^e	-9.66	7.90	0.778	

HRV indicates heart rate variability; SDNN indicates standard deviation of all NN intervals (units ms); TP, total power (ms²); HF, high frequency power (ms²); LF, low frequency power (ms²).

^aadjusted for gender, age, age squared, BMI, BMI squared, smoking status, environmental tobacco smoke exposure, alcohol consumption, physical activity, high-sensitivity C-reactive protein, uric acid levels, hypertension, heart disease, diabetes, street and railway noise, seasonal effects and area.

^bp-values of genotype-specific main effects of the IL6-174G/C polymorphism and TPM₁₀ on HRV (codominant genetic model).

^cp-values of main effects of the IL6-174G/C polymorphism on HRV indices were tested for additive, dominant and recessive genetic models; p-values of the most significant mode of inheritance are presented (¹additive or ²dominant).

^dper 1 µg/m³ TPM₁₀ increase.

^ecompared to reference genotype G/G.

doi:10.1371/journal.pone.0104978.t002

Table 3. Adjusted^a estimates of the mean percent difference of HRV associated with a 1 $\mu\text{g}/\text{m}^3$ increase in average exposure to traffic-related PM₁₀^b by *IL6*-174 G/C genotypes (N = 1549).

HRV parameters	<i>IL6</i> -174 G/C	Estimate ^a	95% CI	$P_{\text{TPM}_{10}}$ (by genotype) ^c	$P_{\text{interaction}}$ (genetic model) ^d
SDNN	GG	-1.77	-3.51	0.01	0.028 (1)
	GC	1.06	-0.47	2.62	
	CC	0.73	-1.62	3.14	
Total Power (TP)	GG	-3.34	-7.22	0.71	0.177 (1)
	GC	2.54	-1.00	6.20	
	CC	-1.00	-6.22	4.51	
Low Frequency Power (LF)	GG	-5.70	-10.36	0.81	0.049 (2)
	GC	1.51	-2.87	6.09	
	CC	-4.98	-11.00	1.45	
High Frequency Power (HF)	GG	-0.16	-6.36	6.45	0.671 (1)
	GC	2.70	-2.84	8.55	
	CC	-3.83	-11.53	4.54	
LF/ HF Ratio	GG	-5.82	-9.88	1.57	0.070 (2)
	GC	-1.51	-5.24	2.37	
	CC	-1.54	-6.98	4.21	

HRV indicates heart rate variability; SDNN indicates standard deviation of all NN intervals (units ms); TP, total power (ms^2); HF, high frequency power (ms^2); LF, low frequency power (ms^2).

^aadjusted for gender, age, age squared, BMI, BMI squared, smoking status, environmental tobacco smoke exposure, alcohol consumption, physical activity, high-sensitivity C-reactive protein, uric acid levels, hypertension, heart disease, diabetes, street and railway noise, seasonal effects and area.

^bAnnual average over 10 years previous to the study.

^cp-values for the genotype-specific TPM₁₀ effect estimate.

^dp-values of interaction effects of the *IL6*-174 G/C polymorphism with TPM₁₀ on HRV were tested for additive, dominant and recessive genetic models; p-values of the most significant mode of inheritance are presented (additive¹ or dominant²).

doi:10.1371/journal.pone.0104978.t003

prevalence of some genotypes limited statistical power of the explanatory analyses. Calculations indicate we had sufficient power to observe a main effect and a TPM₁₀ interaction effect for the candidate SNP *IL6*-174 G/C on SDNN of the order of magnitude reported or larger. These power calculations assumed different HRV parameters to not be independent. Second, we did not have information about IL-6 serum levels and cannot draw conclusions on how the polymorphisms studied influenced the circulating IL-6 levels and finally the cardiovascular health of the SAPALDIA participants. But the *IL6*-174 G/C SNP was associated with higher high sensitive C-reactive protein (mg/l) concentrations in the blood (GG: 2.8; GC: 2.5; CC: 2.4). Third, data on short-term TPM₁₀ was not available, and hence short-term exposure was assessed using averaged normal PM₁₀ from fixed monitoring station measurements. We had obtained 24-hr ECG recordings only once for each participant and were therefore not able to look at longitudinal changes in HRV and its association with the incidence of cardiovascular diseases. Fourth, we had previously reported that the association between TPM₁₀ and HRV is restricted to persons reporting intake of angiotensin converting enzyme inhibitors (ACEI) [10]. The now reported interaction between the *IL6*-174G/C polymorphism and TPM₁₀ remained unchanged after exclusion of participants on ACEI, but we lacked the statistical power for formal assessment of 3-way interactions between TPM₁₀, *IL6* variants and ACEI. Finally, we were unable to adjust our analysis for some short-term factors influencing HRV, such as physical exercise. However, physical exercise at the time of heart rate variability measurement is unlikely to be associated with chronic exposure to TPM₁₀ and thus unlikely to confound the associations reported.

Conclusions

In summary, this cross-sectional study from the general population provides supportive evidence that genetic variation in one of the major proinflammatory cytokines, *IL6*, alters HRV and its association with long-term exposure to traffic-related particulate air pollution. Our findings contribute to the research effort to pinpoint biologic mechanisms mediating air pollution related broad health effects. While the results guide future research efforts to use *IL6* as an important candidate gene in studies of HRV and air pollution, they essentially need replication in different study populations, in longitudinal studies, for different air pollution exposure metrics, and with genetic variant information derived from deep sequencing of the *IL6* gene region.

Acknowledgments

The SAPALDIA group involves more than 50 people that have supplied data and given minor contributions to the interpretation of the results. We would like to thank the whole SAPALDIA team (Acknowledgements S1 in File S1) for their contribution to the study. Additionally, the study could not have been done without the help of the study participants, technical and administrative support and the medical teams and field workers at the local study sites.

Author Contributions

Conceived and designed the experiments: NPH. Performed the experiments: NPH MA MI EB FK MI DC JMG HCP NK ES. Analyzed the data: MA MI EB NPH. Contributed reagents/materials/analysis tools: NPH. Wrote the paper: NPH MA MI EB FK MI DC JMG HCP NK ES. Directly participated in the planning, execution, or analysis of the study and read, revised and approved the manuscript: NPH MA MI EB FK MI DC JMG HCP NK ES.

References

- Bigger JT Jr, Fleiss JL, Rolnitzky LM, Steinman RC (1993) Frequency domain measures of heart period variability to assess risk late after myocardial infarction. *J Am Coll Cardiol* 21: 729–736.
- Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, et al. (2010) Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation* 121: 2331–2378.
- Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ (1987) Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol* 59: 256–262.
- Dekker JM, Crow RS, Folsom AR, Hannan PJ, Liao D, et al. (2000) Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the ARIC Study. *Atherosclerosis Risk In Communities*. *Circulation* 102: 1239–1244.
- Nolan J, Batin PD, Andrews R, Lindsay SJ, Brooksby P, et al. (1998) Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart). *Circulation* 98: 1510–1516.
- Link MS, Dockery DW (2010) Air pollution and the triggering of cardiac arrhythmias. *Curr Opin Cardiol* 25: 16–22.
- Pope CA 3rd, Hansen ML, Long RW, Nielsen KR, Eatough NL, et al. (2004) Ambient particulate air pollution, heart rate variability, and blood markers of inflammation in a panel of elderly subjects. *Environ Health Perspect* 112: 339–345.
- Pieters N, Plusquin M, Cox B, Kicinski M, Vangronsveld J, et al. (2012) An epidemiological appraisal of the association between heart rate variability and particulate air pollution: a meta-analysis. *Heart* 98: 1127–1135.
- Mills NL, Finlayson AE, Gonzalez MC, Tornqvist H, Barath S, et al. (2011) Diesel exhaust inhalation does not affect heart rhythm or heart rate variability. *Heart* 97: 544–550.
- Adam M, Felber Dietrich D, Schaffner E, Carballo D, Barthelemy JC, et al. (2012) Long-term exposure to traffic-related PM(10) and decreased heart rate variability: is the association restricted to subjects taking ACE inhibitors? *Environ Int* 48: 9–16.
- Felber Dietrich D, Gemperli A, Gaspoz J, Schindler C, Liu LJ, et al. (2008) Differences in heart rate variability associated with longterm exposure to NO2. *Environ Health Perspect* 116: 1357–1361.
- Delfino RJ, Staimer N, Tjoa T, Arhami M, Polidori A, et al. (2010) Associations of primary and secondary organic aerosols with airway and systemic inflammation in an elderly panel cohort. *Epidemiology* 21: 892–902.
- Delfino RJ, Staimer N, Tjoa T, Gillen DL, Polidori A, et al. (2009) Air pollution exposures and circulating biomarkers of effect in a susceptible population: clues to potential causal component mixtures and mechanisms. *Environ Health Perspect* 117: 1232–1238.
- Lampert R, Bremner JD, Su S, Miller A, Lee F, et al. (2008) Decreased heart rate variability is associated with higher levels of inflammation in middle-aged men. *Am Heart J* 156: 759 e751–757.
- Ruckerl R, Greven S, Ljungman P, Aalto P, Antoniadou C, et al. (2007) Air pollution and inflammation (interleukin-6, C-reactive protein, fibrinogen) in myocardial infarction survivors. *Environ Health Perspect* 115: 1072–1080.
- Danesh J, Kaptoge S, Mann AG, Sarwar N, Wood A, et al. (2008) Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med* 5: e78.
- Kaptoge S, Seshasai SR, Gao P, Freitag DF, Butterworth AS, et al. (2014) Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis. *Eur Heart J* 35: 578–589.
- Lee JK, Bettencourt R, Brenner D, Le TA, Barrett-Connor E, et al. (2012) Association between serum interleukin-6 concentrations and mortality in older adults: the Rancho Bernardo study. *PLoS One* 7: e34218.
- Ackermann-Lieblich U, Kuna-Dibbert B, Probst-Hensch NM, Schindler C, Felber Dietrich D, et al. (2005) Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991–2003: Methods and characterization of participants. *Soz Präventivmed* 50: 245–263.
- Martin BW, Ackermann-Lieblich U, Leuenberger P, Kunzli N, Stutz EZ, et al. (1997) SAPALDIA: methods and participation in the cross-sectional part of the Swiss Study on Air Pollution and Lung Diseases in Adults. *Soz Präventivmed* 42: 67–84.
- Electrophysiology NAsPa (1996) Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 93: 1043–1065.
- Felber Dietrich D, Schindler C, Schwartz J, Barthelemy JC, Tschopp JM, et al. (2006) Heart rate variability in an ageing population and its association with lifestyle and cardiovascular risk factors: results of the SAPALDIA study. *Europace* 8: 521–529.
- Liu LJ, Curjuric I, Keidel D, Heldstab J, Kunzli N, et al. (2007) Characterization of source-specific air pollution exposure for a large population-based Swiss cohort (SAPALDIA). *Environ Health Perspect* 115: 1638–1645.
- Kunzli N, Bridevaux PO, Liu LJ, Garcia-Esteban R, Schindler C, et al. (2009) Traffic-related air pollution correlates with adult-onset asthma among never-smokers. *Thorax* 64: 664–670.
- Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, et al. (2001) Interleukin-6 gene $-174g>c$ and $-572g>c$ promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. *Arterioscler Thromb Vasc Biol* 21: 1458–1463.
- Kelberman D, Fife M, Rockman MV, Brull DJ, Woo P, et al. (2004) Analysis of common IL-6 promoter SNP variants and the AnTn tract in humans and primates and effects on plasma IL-6 levels following coronary artery bypass graft surgery. *Biochim Biophys Acta* 1688: 160–167.
- Kormann MS, Carr D, Klopp N, Illig T, Leupold W, et al. (2005) G-Protein-coupled receptor polymorphisms are associated with asthma in a large German population. *Am J Respir Crit Care Med* 171: 1358–1362.
- Imboden M, Nieters A, Bircher A, Brutsche M, Becker N, et al. (2006) Cytokine gene polymorphisms and atopic disease in two European cohorts. (ECRHS-Basel and SAPALDIA). *Clin Mol Allergy* 4: 9.
- Bender R, Lange S (2001) Adjusting for multiple testing—when and how? *J Clin Epidemiol* 54: 343–349.
- Chan KH, Brennan K, You NC, Lu X, Song Y, et al. (2011) Common variations in the genes encoding C-reactive protein, tumor necrosis factor- α , and interleukin-6, and the risk of clinical diabetes in the Women's Health Initiative Observational Study. *Clin Chem* 57: 317–325.
- Ljungman P, Bellander T, Nyberg F, Lampa E, Jacquemin B, et al. (2009) DNA variants, plasma levels and variability of interleukin-6 in myocardial infarction survivors: results from the AIRGENE study. *Thromb Res* 124: 57–64.
- Haensel A, Mills PJ, Nelesen RA, Ziegler MG, Dimsdale JE (2008) The relationship between heart rate variability and inflammatory markers in cardiovascular diseases. *Psychoneuroendocrinology* 33: 1305–1312.
- Lieb DC, Parson HK, Mamikunian G, Vinik AI (2012) Cardiac autonomic imbalance in newly diagnosed and established diabetes is associated with markers of adipose tissue inflammation. *Exp Diabetes Res* 2012: 878760.
- Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, et al. (1998) The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 102: 1369–1376.
- Hulkkonen J, Pertovaara M, Anttonen J, Pasternack A, Hurme M (2001) Elevated interleukin-6 plasma levels are regulated by the promoter region polymorphism of the IL6 gene in primary Sjogren's syndrome and correlate with the clinical manifestations of the disease. *Rheumatology (Oxford)* 40: 656–661.
- Lalouschek W, Schillinger M, Hsieh K, Endler G, Greisneggger S, et al. (2006) Polymorphisms of the inflammatory system and risk of ischemic cerebrovascular events. *Clin Chem Lab Med* 44: 918–923.
- Humphries SE, Luong LA, Ogg MS, Hawe E, Miller GJ (2001) The interleukin-6 -174 G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men. *Eur Heart J* 22: 2243–2252.
- Sie MP, Sayed-Tabatabaie FA, Oei HH, Uitterlinden AG, Pols HA, et al. (2006) Interleukin 6 -174 g/c promoter polymorphism and risk of coronary heart disease: results from the rotterdam study and a meta-analysis. *Arterioscler Thromb Vasc Biol* 26: 212–217.
- Hulkkonen J, Lehtimäki T, Mononen N, Juonala M, Hutri-Kahonen N, et al. (2009) Polymorphism in the IL6 promoter region is associated with the risk factors and markers of subclinical atherosclerosis in men: The Cardiovascular Risk in Young Finns Study. *Atherosclerosis* 203: 454–458.
- Riikola A, Sipilä K, Kahonen M, Jula A, Nieminen MS, et al. (2009) Interleukin-6 promoter polymorphism and cardiovascular risk factors: the Health 2000 Survey. *Atherosclerosis* 207: 466–470.
- Mayosi BM, Avery PJ, Baker M, Gaukrodger N, Imrie H, et al. (2005) Genotype at the -174 G/C polymorphism of the interleukin-6 gene is associated with common carotid artery intimal-medial thickness: family study and meta-analysis. *Stroke* 36: 2215–2219.
- Sie MP, Mattace-Raso FU, Uitterlinden AG, Arp PP, Hofman A, et al. (2008) The interleukin-6-174 G/C promoter polymorphism and arterial stiffness; the Rotterdam Study. *Vasc Health Risk Manag* 4: 863–869.
- Luttman-Gibson H, Suh HH, Coull BA, Dockery DW, Sarnat SE, et al. (2010) Systemic inflammation, heart rate variability and air pollution in a cohort of senior adults. *Occupational and Environmental Medicine* 67: 625–630.
- Zanobetti A, Baccarelli A, Schwartz J (2011) Gene-air pollution interaction and cardiovascular disease: a review. *Prog Cardiovasc Dis* 53: 344–352.
- Park SK, O'Neill MS, Wright RO, Hu H, Vokonas PS, et al. (2006) HFE genotype, particulate air pollution, and heart rate variability: a gene-environment interaction. *Circulation* 114: 2798–2805.
- Ren C, Park SK, Vokonas PS, Sparrow D, Wilker E, et al. (2010) Air pollution and homocysteine: more evidence that oxidative stress-related genes modify effects of particulate air pollution. *Epidemiology* 21: 198–206.
- Probst-Hensch NM, Imboden M, Felber Dietrich D, Barthelemy JC, Ackermann-Lieblich U, et al. (2008) Glutathione S-transferase polymorphisms, passive smoking, obesity, and heart rate variability in nonsmokers. *Environ Health Perspect* 116: 1494–1499.