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Acute air pollution effects on heart rate variability are modified by SNPs involved in cardiac rhythm in individuals with diabetes or impaired glucose tolerance ☆, ☆☆

Regina Hampel [a,*], Susanne Breitner [a,b], Alexandra Schneider [a], Wojciech Zareba [c], Ute Kraus [a,b], Josef Cyrys [a,d], Uta Geruschkat [a], Petra Belcredi [a,e], Martina Müller [f,g,h], H.-Erich Wichmann [b,i], Annette Peters [a], For the Cooperative Health Research in the Region of Augsburg (KORA) Study Group

[a] Institute of Epidemiology II, Helmholtz Zentrum München, Neuherberg, Germany
[b] Institute of Biometrics and Epidemiology, Ludwig-Maximilians-University of Munich, Munich, Germany
[c] Cardiology Division, University of Rochester Medical Center, Rochester, United States of America
[d] ESC-Environmental Science Center, University of Augsburg, Augsburg, Germany
[e] Hospital of Augsburg, MONICA/KORA Myocardial Infarction Registry, Augsburg, Germany
[f] Department of Medicine I, University Hospital Großhadern, Ludwig-Maximilians-University of Munich, Germany
[g] Institute of Medical Informatics, Biometry and Epidemiology, Chair of Epidemiology and Chair of Genetic Epidemiology, Ludwig-Maximilians-University of Munich, Munich, Germany
[h] Institute of Genetic Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany
[i] Institute of Epidemiology I, Helmholtz Zentrum München, Neuherberg, Germany

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ABSTRACT

Background: Epidemiological studies have shown associations between particulate matter (PM) and heart rate variability (HRV).
Objectives: We investigated the effects of air pollution on the root mean square of successive differences (RMSSD) and the standard deviation of normal-to-normal intervals (SDNN) and effect modifications by single nucleotide polymorphisms (SNP).
Methods: Between March 2007 and December 2008 207 ECG recordings comprising 1153 1 h-intervals were measured in 61 individuals with type 2 diabetes or impaired glucose tolerance (IGT) from Augsburg, Germany. Associations between 1 h-averages of air pollutants (PM, sulphate, black carbon, and ultrafine particles) and ECG parameters were analyzed using additive mixed models. Genotypes of 139 SNPs supposed to be involved in cardiac rhythm were identified in the literature. Using regression trees for longitudinal data, SNPs associated with ECG parameters were determined and included as potential air pollution effect modifiers.
Results: We observed concurrent and lagged decreases in SDNN by about 2–5% in association with all air pollutants, especially in participants with at least one minor allele of rs332229. Increases in PM <2.5 μm ($PM_{2.5}$) were associated with 4 h-lagged decreases of -6.6%[95%-confidence interval: -10.6; -2.6%] and $-13.0\%[-20.7;-5.1\%]$ in SDNN in individuals with one or two minor alleles. We observed a -7.2%

Abbreviations: AIC, akaike information criterion; BC, black carbon; *CHT1*, choline transporter gene; CI, confidence interval; T2D, type 2 diabetes; ECG, electrocardiogram; GWAS, genome-wide association study; HR, heart rate; HRV, heart rate variability; HWE, Hardy–Weinberg equilibrium; IGT, impaired glucose tolerance; IQR, interquartile range; PM, particulate matter; PM_{10}, PM with an aerodynamic diameter below 10 μm; $PM_{2.5}$, pM with an aerodynamic diameter below 2.5 μm; PNC, Particle number concentration; RMSSD, root mean square of successive differences; SNP, single nucleotide polymorphism; SDNN, standard deviation of normal-to-normal intervals; UFP, ultrafine particles

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* Correspondence to: Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany. Fax: +49 89 3187 3380.

E-mail addresses: regina.hampel@helmholtz-muenchen.de (R. Hampel), susanne.breitner@helmholtz-muenchen.de (S. Breitner), alexandra.schneider@helmholtz-muenchen.de (A. Schneider), Wojciech_Zareba@URMC.Rochester.edu (W. Zareba), ute.kraus@helmholtz-muenchen.de (U. Kraus), cyrys@helmholtz-muenchen.de (J. Cyrys), uta.geruschkat@helmholtz-muenchen.de (U. Geruschkat), belcredi@helmholtz-muenchen.de (P. Belcredi), martina.mueller@helmholtz-muenchen.de (M. Müller), wichmann@helmholtz-muenchen.de (H.-E. Wichmann), peters@helmholtz-muenchen.de (A. Peters).

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[−12.2; −1.8%] reduction in RMSSD associated with concurrent increases in $PM_{2.5}$. Individuals with at least one minor allele of rs2096767 or at most one minor allele of rs2745967 exhibited stronger $PM_{2.5}$ effects. *Conclusions:* We identified a genetic predisposition in persons with diabetes or IGT making them potentially more susceptible to air pollutants with regard to changes in HRV.

1. Introduction

Initially, Pope et al. (1999), Peters et al. (1999), and Gold et al. (2000) reported associations between elevated air pollution levels and increases in heart rate (HR) and decreases in heart rate variability (HRV). Recent studies were able to confirm changes in heart rhythm. For instance, researchers observed decreased time and frequency domain parameters in association with elevated air pollution levels in patients with coronary artery disease (CAD) (Zanobetti et al., 2010), in individuals with metabolic syndrome (Min et al., 2009; Park et al., 2010), and in healthy participants (Wu et al., 2010). It is assumed that the observed findings might be a consequence of (1) an imbalance of the autonomic nervous system, (2) a direct involvement of the electric system of the heart, or (3) a systemic inflammation and oxidative reaction promoting vascular dysfunction (Brook et al., 2010; Pope and Dockery, 2006).

Individuals with type 2 diabetes (T2D) are reported to be a susceptible population. Peel et al. (2007) and Zanobetti and Schwartz (2001), for example, observed an increased risk for emergency department visits and hospital admissions in association with air pollution increases in diabetes patients, respectively. Diabetes can cause autonomic neuropathy leading to a reduced HRV (Kudat et al., 2006). Accordingly, Park et al. (2005) observed a stronger reduction in some HRV parameters in association with particulate air pollution increases in T2D individuals compared to people without T2D.

Ambient air pollution may act on the autonomic function via oxidative stress pathways (Brook et al., 2010). Therefore, not only people with T2D might be susceptible to air pollution but also people with genetic predispositions related to these pathways. Accordingly, authors observed modifications of air pollution effects on HRV parameters by oxidative stress-related single nucleotide polymorphisms (SNP) (Chahine et al., 2007; Schwartz et al., 2005).

It has also been reported that HR and HRV (Eijgelsheim et al., 2010; Newton-Cheh et al., 2007) as well as repolarization parameters (Chambers et al., 2010; Pfeufer et al., 2009) are modulated by variants in genes. We hypothesized that people with an altered cardiac rhythm due to genetic predispositions might also show different reactions to air pollution exposure. Therefore, we investigated the modification of air pollution effects on HRV parameters by SNPs involved in cardiac rhythm in potentially susceptible participants with T2D or impaired glucose tolerance (IGT) indicating an enhanced risk for T2D. In contrast to already published studies (Chahine et al., 2007; Probst-Hensch et al., 2008; Schwartz et al., 2005), which used single candidate SNPs as potential effect modifiers we performed, in a first step, regression trees for longitudinal data (Sela and Simonoff, 2010) in order to identify SNPs with an influence on repeated measurements of ECG parameters. Only these influential SNPs were then used as potential effect modifiers. The main advantage of this procedure was that we reduced the number of performed tests. Moreover, with this method we were able to select more than 130 SNPs from published genome-wide association studies (GWAS) and did not have to restrict our analysis to only a few candidate SNPs.

2. Materials and methods

2.1. Study design and study population

As part of the University of Rochester Particulate Matter Center investigations, a prospective panel study was conducted between March 19 2007 and December 17 2008 in Augsburg, Germany. Individuals with T2D or IGT were recruited from the KORA (Cooperative Health Research in the Region of Augsburg) F4 cohort, which was conducted in the years 2006–2008 and also serves as study sample for genome-wide analysis (Holle et al., 2005; Wichmann et al., 2005). TD2 was either physician-diagnosed or by medication use. All other participants had an oral glucose tolerance test (OGTT). Participants with a fasting glucose level >125 mg/dl or a 2 h OGTT glucose level ≥ 200 mg/dl were also defined as having TD2. IGT was defined as 2 h OGTT glucose levels ≥ 140 mg/dl but <200 mg/dl. In our study, all individuals participated in up to four repeated ECG recordings scheduled every 4–6 weeks on the same weekday and at the same time of the day. Data on health status, medication as well as disease and smoking history were gathered at a baseline visit. Exclusion criteria were current smoking, intake of platelet aggregation inhibitors except for acetylsalicylic acid, a myocardial infarction (MI) and/or interventional procedure (PTCA, bypass surgery) less than six months before the start of the study, chronic inflammatory diseases such as Crohn's disease, ulcerative colitis, or rheumatoid arthritis, an implanted pacemaker, atrial fibrillation, allergy to latex, and thrombosis or shunt in an arm. All participants gave written informed consent and the study protocol was approved by the Ethics Commission of the Bavarian Chamber of Physicians ("Bayerische Landesaerztekammer").

2.2. Clinical measurements

Participants were equipped with a 12-lead Mortara H12 digital Holter recorder (Mortara Instrument, Milwaukee, WI, USA). They left the study center to pursue their daily routines and returned after four to six hours. ECG parameters such as HR, repolarization, and HRV time and frequency domain parameters were determined on an hourly basis. Therefore, repeated ECG recordings for each participant and repeated 1 h-averages of ECG parameters within one recording were available. Only individuals with at least one ECG recording with a duration of at least two hours were used for analysis. In our study, HR, standard deviation of normal-to-normal intervals (SDNN, i.e. square root of variance), and root mean square of successive differences (RMSSD) were the outcomes of interest.

2.3. Genotyping

Genome-wide data were determined based on MACH imputation of Affymetrix 6.0 genotyped data (see Supplementary material). For our analysis we only used SNPs supposed to be involved in cardiac rhythm (e.g. HR, RMSSD, high and low frequency, and PR- and QT-interval), which were already identified in the literature and published before August 2010. For the literature research in the database *PubMed* we used keywords such as *gene, genetic, heart rate variability, repolarization, T-wave, QT-interval, RMSSD, SDNN, frequency domain,* and *time domain.* SNPs were coded counting the number of minor alleles. We tested for evidence against the additive genetic model using a procedure introduced by Schaid (2004). For our analysis we excluded SNPs in case of a minor allele frequency below 5%, an imputation quality (observed vs. expected variance of the genotypes) below 0.6, or a significant deviation from the additivity assumption. See Supplementary material for a more detailed description of the genetic data.

2.4. Air pollution and meteorology data

Amongst others, hourly means of air temperature, relative humidity, barometric pressure, particulate matter (PM) with an aerodynamic diameter below 10 μm or 2.5 μm (PM_{10}, $PM_{2.5}$), ultrafine particles (UFP) with a size range of 0.01–0.1 μm in diameter, and sulfate and black carbon (BC) mass concentration of $PM_{2.5}$ were measured at a central measurement site in Augsburg throughout the complete study period as described previously (Cyrys et al., 2008; Pitz et al., 2008). For a more detailed description of the air pollution measurement and the replacement of missing values see Supplementary material.

2.5. Statistical analysis

2.5.1. Influential SNPs

In a first step, we conducted a literature research and identified SNPs which have already been shown to modulate repolarization and HRV parameters. In order to determine the influence of SNPs on ECG parameters we used regression trees for longitudinal data implemented in the R package REEMtree (Sela and Simonoff, 2010). This method alternates between estimating the regression tree, assuming that the previously estimated random effects of a mixed model are

correct, and estimating the random effects, using the information of the regression tree performed in the prior step. In general, a regression tree is a non-parametric method, which performs binary recursive partitioning dividing the outcome variable in homogeneous subgroups using covariable information (Breiman et al., 1983). For each ECG parameter we estimated regression trees for longitudinal data always excluding one single ECG recording in order to check the robustness of the trees. SNPs, which occurred at least in 75% of all trees, were used to assess a potential modification of the air pollution effect.

2.5.2. Air pollution effects

Air pollution effects were estimated with SAS statistical package (version 9.2; SAS Institute Inc., Cary, NC, USA) using additive mixed models with a random participant effect. A first order autoregressive covariance structure has been used to account for the dependencies between the repeated ECG recordings.

A confounder selection was conducted for each ECG parameter separately. Potential confounders were long-term time trend, time of the day, day of the week, air temperature, relative humidity, and barometric pressure. Possible lags considered for meteorology were the 1 h-averages concurrent to the 1 h-ECG recordings, 1 h-averages 1 h up to 12 h, and 24 h-averages 0–23 h and 24–47 h before each 1 h-ECG interval. The confounders were included linearly or smoothly as penalized splines (P-Splines) to allow for a non-linear relationship. The lag and shape, which minimized the Akaike Information Criterion (AIC) was selected. If a confounder was included as P-Spline, we checked whether a polynomial led to a smaller AIC. Barometric pressure, time of the day, and day of the week were only selected in case of model fit improvement.

After assessing the confounder model, 1 h-averages of air pollutants concurrent to the 1 h-averages of ECG measurements and up to 6 h before the ECG recordings were separately added to the confounder model and the effects were estimated linearly. In a further analysis, influential SNPs identified with regression trees were used as air pollution effect modifiers. Additionally, main effects and effect modifications on ECG parameters were calculated for participants with T2D and IGT separately. With this subgroup analysis we intended to study the impact of air pollutants in subjects with an enhanced risk for T2D but who were not treated by beta-blockers, statins, or anti-diabetic medications as these medications may modify the effects of particle exposures.

2.5.3. Sensitivity analyses

As sensitivity analysis air pollution effects were estimated smoothly as P-Splines in order to check the linearity of the relationship between air pollutants and ECG parameters. Furthermore, we excluded ECG recordings of occasional smokers and of participants who reported that they have been exposed to environmental tobacco smoke (ETS) during the recording. We also excluded individuals with CAD, angina pectoris, or a MI. Additionally, we only included participants without intake of beta-adrenergic receptor blockers (beta-blockers).

3. Results

3.1. Study population and clinical measurements

76 subjects with T2D or IGT were willing to participate in our study including ECG recordings. We excluded one smoker, three individuals with a chronic inflammatory disease, four individuals with atrial fibrillation, two persons because of physical constraints and one individual each with an implanted pace maker or latex allergy. Further, three participants did not give consent in genotyping. Overall, 61 participants with 207 valid ECG recordings (average duration: 5.6 h) comprising 1153 1 h-intervals were available for analysis. Table 1 describes the baseline characteristics of the non-smoking participants. Patient characteristics were compared between participants with T2D and IGT using a Student's *t*-test for continuous variables and a χ^2-test for categorical variables. Fisher's

Table 1
Description of the study population of 61 participants with type 2 diabetes or impaired glucose tolerance.

	All (N=61)		**Diabetes (N=31)**		**IGT (N=30)**		***p*-value**
	Mean	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	
Age (years)	65.7	(8.0)	66.7	(6.7)	64.8	(9.2)	0.37[a]
BMI (kg/m^2)	30.2	(4.7)	30.9	(4.4)	29.5	(4.9)	0.27[a]
	N	**(%)**	***N***	**(%)**	***N***	**(%)**	
Age (years)							
≤ 60	14	(23)	6	(19)	8	(27)	0.50[b]
> 60	47	(77)	25	(81)	22	(73)	
BMI (kg/m^2)							
≤ 30	32	(52)	15	(48)	17	(57)	0.52[b]
> 30	29	(48)	16	(52)	13	(43)	
Gender							
Male	40	(66)	23	(74)	17	(57)	0.15[b]
Female	21	(34)	8	(26)	13	(43)	
Smoking							
Never smoker	26	(43)	10	(32)	16	(53)	0.16[c]
Ex smoker	34	(58)	20	(65)	14	(47)	
Occasional smoker	1	(2)	1	(3)	0	(0)	
HbA1c							
< 6.5%	46	(75)	17	(55)	29	(97)	< 0.0001[c]
≥ 6.5%	15	(27)	14	(45)	1	(3)	
History of							
Coronary heart disease	4	(7)	3	(10)	1	(3)	0.61[c]
Angina pectoris	5	(8)	2	(6)	3	(10)	0.67[c]
Myocardial infarction	6	(10)	5	(16)	1	(3)	0.20[c]
Hypertension	40	(66)	20	(65)	20	(67)	0.86[b]
Medication use							
Antidiabetics	18	(30)	17	(55)	1	(3)	< 0.0001[c]
Beta-adrenergic receptor blockers	18	(30)	11	(35)	7	(23)	0.30[b]
Statins	12	(20)	10	(32)	2	(7)	0.02[c]

IGT: impared glucose tolerance, SD: standard deviation, BMI: body mass index, HbA1c: glycosylated hemoglobin A1c.

[a] Student's *t*-test.
[b] Chi-square test.
[c] Fisher's exact test.

Table 2
Description of the 1 h-averages of ECG parameters.

	N	Mean	SD	Min	25%	Median	75%	Max	IQR
HR (beats/min)	1152[a]	79.3	14.4	46.5	69.2	78.9	87.9	132.9	18.7
RMSSD (ms)	1153	34.2	32.3	1.3	16.2	22.7	36.7	227.3	20.5
SDNN (ms)	1153	76.6	27.2	11.8	56.1	74.2	94.6	161.2	38.4

[a] One 1 h-interval was excluded because of a large residual in the mixed model analysis, HR: heart rate, RMSSD: root mean square of successive differences, SDNN: standard deviation of all normal-to-normal intervals, SD: standard deviation, IQR: interquartile range.

exact test was used if one category contained less than five observations. Characteristics of participants with T2D and IGT did not differ except for glycosylated hemoglobin A1c, intake of antidiabetic medication, and intake of statins. Table 2 shows a description of the analyzed ECG parameters. Spearman correlation coefficients between ECG parameters were calculated for each ECG recording separately. According to the median of these correlation coefficients all ECG parameters were uncorrelated ($|r| < 0.5$). HR, RMSSD, and SDNN did not differ significantly between participants with T2D or IGT (data not shown).

3.2. Air pollution and meteorological data

Air pollutants and meteorological variables are described in Supplementary material, Table 2. 1 h-averages of PM_{10} and $PM_{2.5}$ were highly correlated with each other ($r=0.9$) as well as with BC and sulfate ($r=0.7$) but not with UFP ($r<0.5$). BC, sulfate, and UFP were not or only moderately correlated ($r<0.6$) Supplementary material, Fig. 1 shows the time pattern of the air pollutants during the study period.

3.3. Genotyping and influential SNPs

We identified 139 SNPs in the literature, which are reported to have an influence on repolarization and HRV parameters. In our data, seven SNPs were excluded either due to a minor allele frequency $<5\%$ or an imputation quality <0.6. Seven, eight, and ten SNPs did not have an additive effect on HR, RMSSD, and SDNN, respectively, and therefore were not used for the regression tree analysis.

Seven, fourteen, and eleven SNPs were chosen using the tree selection procedure for HR, RMSSD, and SDNN, respectively. Supplementary material, Table 1contains information about the selected SNPs such as the position, minor allele frequency, the authors who initially reported modulations of ECG parameters by these SNPs, and their main effects on ECG parameters estimated with mixed models in our study. The regression trees for HR, SDNN, and RMSSD are given in the Supplementary material, Fig. 2.

3.4. Main effects of air pollutants

Fig. 1 shows the percent changes of the mean ECG parameters per interquartile range (IQR: difference between the third and first quartile) increase in air pollutants together with 95%-confidence intervals (CI). Increased BC levels were only marginally associated with an increase in HR (percent change: 0.9%, 95%–CI: [0.0;1.8%]) with a lag of 6 h. We observed an association between increases in PM_{10} and $PM_{2.5}$ and a concurrent reduction in RMSSD (-5.3% $[-9.3;-1.1\%]$ and $-7.2\%[-12.2;-1.8\%]$, respectively). Furthermore, RMSSD changed by $-3.8\%[-7.1;-0.5\%]$ and -5.2% $[-9.8;-0.4\%]$ in association with elevated BC levels with a lag of 1 h and 6 h, respectively. Elevated $PM_{2.5}$ level led to concurrent ($-3.3\%[-6.0;-0.7]$) and lagged decreases in SDNN by about

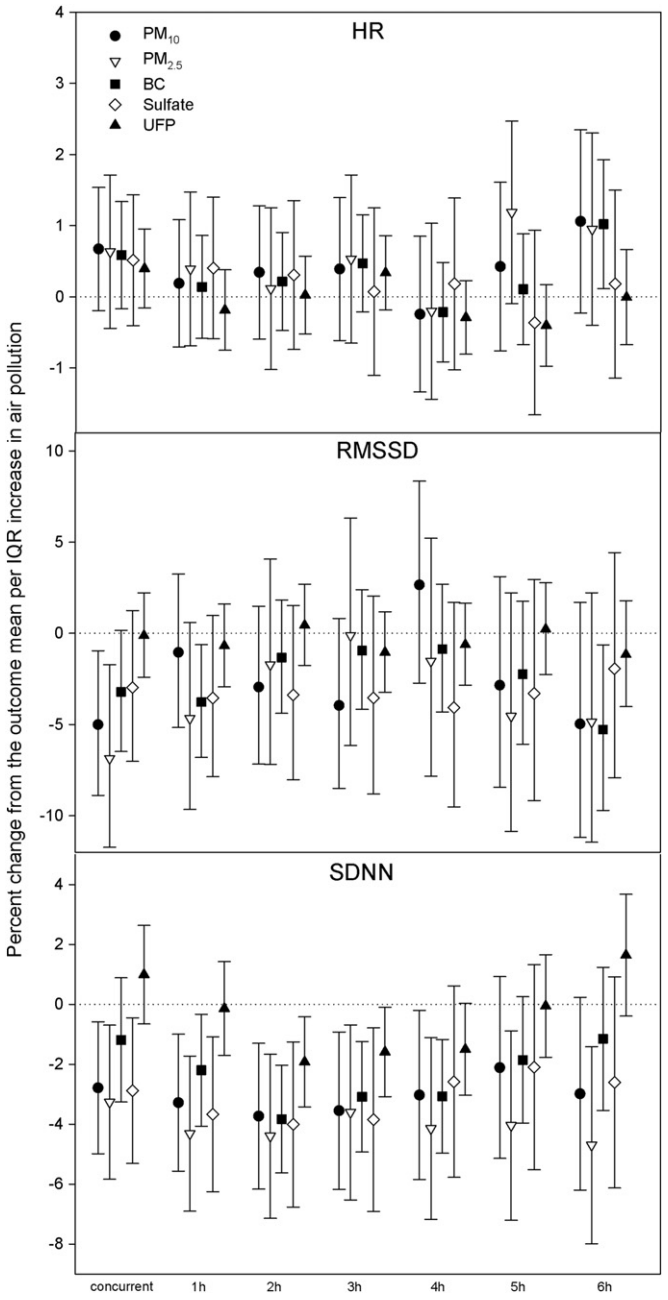

Fig. 1. Concurrent effects of 1 h-averages of air pollutants on 1 h-averages on heart rate (HR), root mean square of successive differences (RMSSD), and standard deviation of all normal-to-normal intervals (SDNN).

3–4%. We observed similar effects of PM_{10} and sulfates. Increases in BC and UFP were only related with lagged decreases in SDNN showing the strongest associations with a lag of 2 h (-3.7% $[-5.6;-1.8\%]$ and $-1.9\%[-3.4;-0.4\%]$, respectively).

3.5. Effect modification

We observed no significant air pollution effects on HR; therefore, we did not calculate effect modifications by SNPs for this ECG parameter. As $PM_{2.5}$ showed the strongest main effects on ECG parameters and PM variables were highly correlated with BC and sulfate but uncorrelated with UFP, we only present $PM_{2.5}$ and UFP effect modifications by SNPs.

rs333229 was the strongest effect modifier on SDNN (Fig. 2). 34 participants with 651 1 h-ECG recordings were homozygous

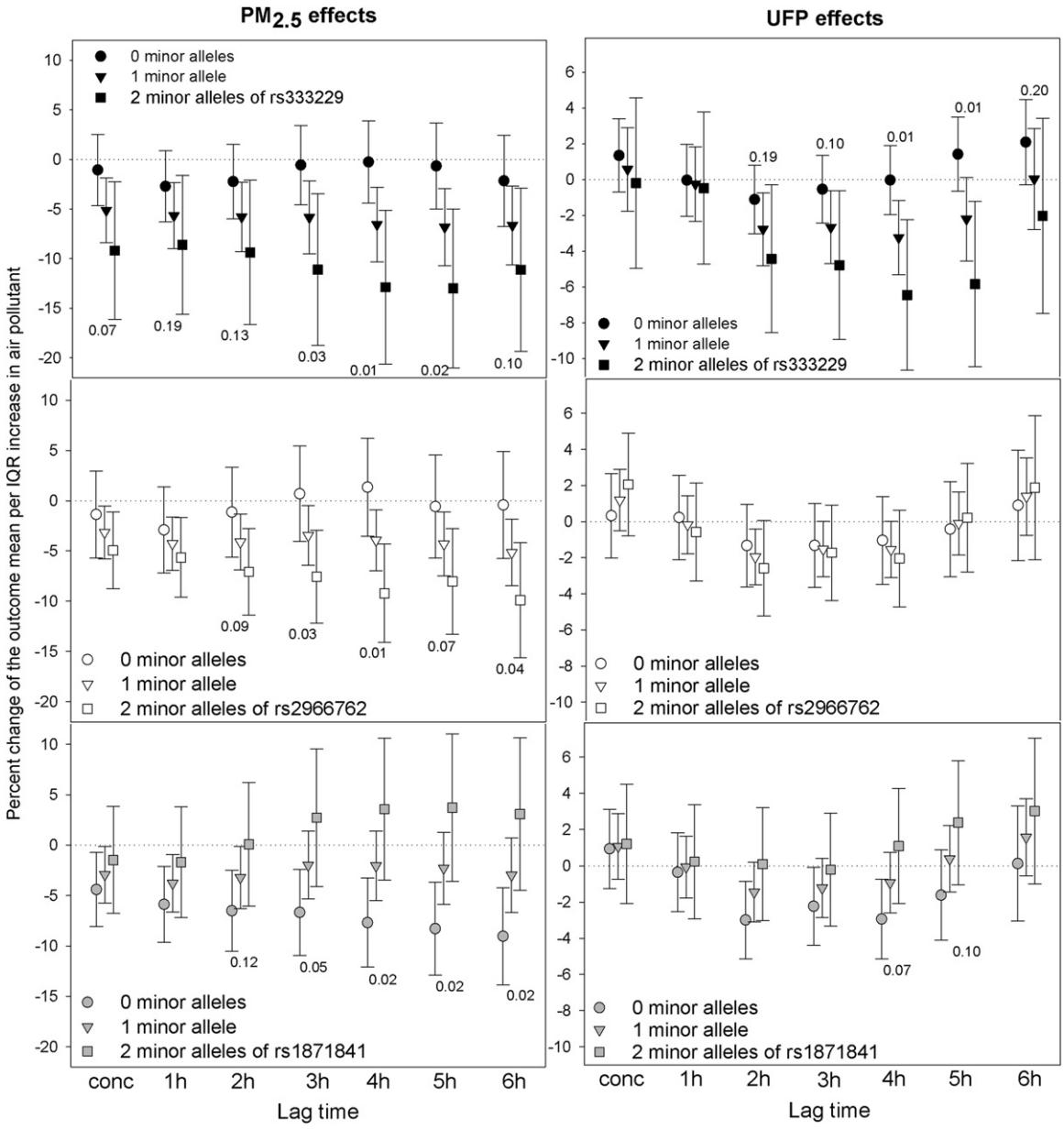

Fig. 2. Effects of $PM_{2.5}$ and UFP on SDNN modified by the number of minor alleles of rs333229, rs2966762, and rs1871841. *P*-values of interaction <0.2 are indicated.

carriers of the major allele, 23 participants with 429 1 h-ECG recordings were heterozygous, and 4 participants with 73 1 h-ECG recordings were homozygous carriers of the minor allele. Throughout all lags only participants with at least one minor allele showed a reduction in SDNN in association with increases in $PM_{2.5}$. Individuals with no minor allele did not react to elevated $PM_{2.5}$ levels. We observed the strongest modification with a lag of 4 h (*p*-value of interaction=0.01). Participants with one and two minor alleles exhibited a $-6.6\%[-10.3;-2.8\%]$ and a -12.9% $[-20.6;-5.1\%]$ decreased SDNN, respectively. $PM_{2.5}$ effect modification by rs2966762 resulted in a similar pattern with weaker effects (Fig. 2). Borderline and significant interaction effects between rs333229 and UFP on SDNN were detected with a lag of 2 h to 5 h. SDNN decreased by about 2–3% in individuals with one and about 4–6% in individuals with two minor alleles. Furthermore, an increase in $PM_{2.5}$ led to a 4–8% and to a 2–4% decrease in SDNN in participants with no or one minor allele of rs1871841 (Fig. 2). The concurrent response of RMSSD to increases in $PM_{2.5}$ was modified by rs2096767 and rs2745967. Elevated $PM_{2.5}$ levels led to a $-10.0\%[-15.5;-4.1\%]$ and a $-13.2\%[-23.3;-1.8\%]$ reduction in RMSSD in individuals with one and two minor alleles of rs2096767, respectively. In contrast, people with no (-13.2% $[-20.3;-5.6\%]$) and one minor allele ($-6.4\%[-11.5;-1.0\%]$) in rs2745967 exhibited a decrease in RMSSD in association with $PM_{2.5}$. We observed no effect modification by other SNPs selected with regression trees.

Analyzing air pollution effects on RMSSD and SDNN for participants with D2TM and IGT separately showed that significant main and interaction effects were only observed in individuals with IGT. Fig. 3 illustrates the $PM_{2.5}$ and UFP effect modification by rs333229 on SDNN in the two panels. An increase in $PM_{2.5}$ levels led to a 11–15% and to a 12–20% reduction in SDNN in participants with IGT having one or two minor alleles of rs333229, respectively. SDNN was reduced to a smaller amount (3–8%) in association with increases in UFP with a lag of 2 h to 5 h in individuals with IGT with at least one minor allele. We also observed effect modifications by rs2966762 and rs1871841 on SDNN only in participants with IGT (data not shown). No significant air pollution effects and effect

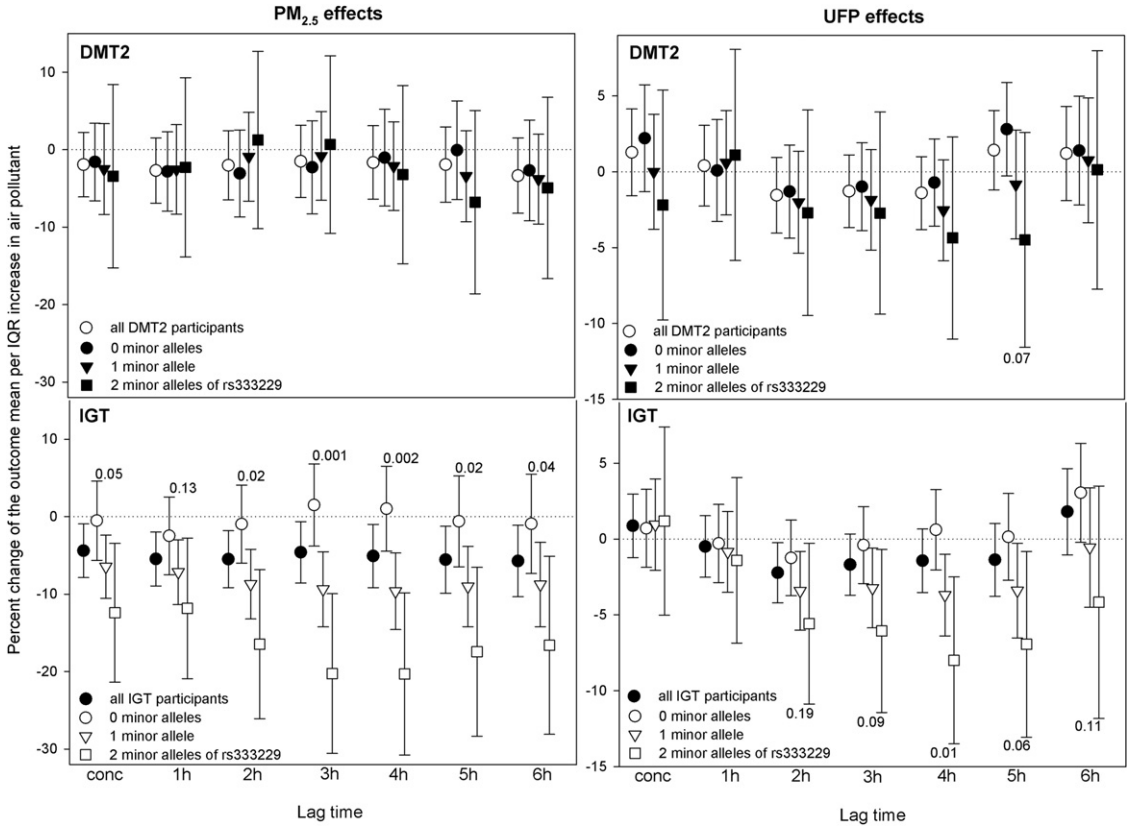

Fig. 3. Effects of $PM_{2.5}$ and UFP on SDNN modified by the number of minor alleles of rs333229 for T2D and IGT participants separately. *P*-values of interaction <0.2 are indicated.

modifications by SNPs were detected on SDNN and RMSSD in participants with T2D.

3.6. Sensitivity analyses

There was no evidence for a deviation from linearity of the relationship between air pollutants and ECG parameters. Excluding 3 ECG recordings of one occasional smoker and 17 ECG recordings of 14 participants who have been exposed to ETS during the recording did not result in notable changes in the air pollution effects of both the main analysis and the interaction analysis. The exclusion of 18 participants (66 ECG recordings) with an intake of beta-blockers and of 11 participants (37 ECG recordings) with CAD, angina pectoris, or MI led to similar but less significant air pollution effects on SDNN (data not shown).

4. Discussion

4.1. Summary

We observed concurrent and lagged decreases in SDNN in association with elevated 1 h-averages of PM and UFP levels in participants with metabolic disorders and especially in individuals with at least one minor allele of rs333229. Weaker effect modifications were observed for rs2966762 and rs1871841. A reduction in RMSSD was only associated with concurrent PM increases. These PM effects were stronger in individuals with at least one minor allele of rs2096767 or at most one minor allele of rs2745967. In general, air pollution effects seemed to be more pronounced in individuals with IGT than with T2D. We observed no air pollution effects on HR.

4.2. Air pollution and HRV

Previous studies assessed changes in HRV in association with air pollution exposure calculated as 4 h-, 6 h-, or 24 h-averages (Luttmann-Gibson et al., 2006; Schneider et al., 2010). Zanobetti et al. (2010) observed a $-1.5\%[-2.5;-0.4\%]$ decrease in RMSSD but no changes in SDNN in association with elevated 1 h-averages of $PM_{2.5}$ directly preceding the ECG recording in patients with CAD. In our study, we observed a concurrent reduction in RMSSD by $-6.8\%[-11.7;-1.7\%]$ and in SDNN by $-3.3\%[-5.8;-0.7\%]$. However, BC effects were less pronounced in our study compared to the findings of Zanobetti et al. Similar to our findings, a study conducted in taxi drivers in Beijing detected a $-2.2\%[-3.8;-0.6\%]$ reduction in SDNN in association with increases in 30 min-averages of $PM_{2.5}$ measured inside the taxicab (Wu et al., 2010). Furthermore, a study by Adar et al. (2007) showed that increases in 1 h-concentrations of traffic-related $PM_{2.5}$ led to a decreased SDNN and RMSSD in elderly participants. In contrast to our study, they also observed an increase in HR associated with elevated $PM_{2.5}$ levels. However, these studies only reported the air pollution effects directly preceding the ECG recording.

Our findings of a rapid decrease in HRV in association with elevated PM-levels might be mediated by a perturbation of the balance of the systemic autonomic nervous system due to a stimulation of lung receptors or nerve endings in the human airways by inhaled particles (Brook et al., 2010). RMSSD is an index of parasympathetic modulation, whereas SDNN reflects the variability of both sympathetic and parasympathetic activity. As we observed a stronger reduction in SDNN than in RMSSD in association with PM increases we assume that the activation of the sympathetic nervous system is more pronounced than the vagal withdrawal. However, we did not find air pollution effects on HR, an ECG parameter reflecting sympathetic activation. Additionally, we found a decrease in SDNN

associated with elevated UFP levels. A small fraction of UFP may pass alveolar walls and affect the electric system of the heart directly (Peters et al., 2006), which might lead to a reduced HRV. We detected concurrent and delayed PM effects but only lagged UFP effects on SDNN. Therefore, we hypothesize that different effects of PM and UFP might reflect different biological pathways activated by different particle properties.

It has also been shown that a reduced HRV might be a precursor of cardiovascular problems (Buccelletti et al., 2009; Lanza et al., 2006; Reed et al., 2005). Hence, our observed findings might be an intermediate step linking air pollution exposure to cardiovascular health.

4.3. Genotypes and susceptibility to air pollution

Susceptibility to air pollution exposure might be partly affected by genetic predispositions as studies reported a modified HRV response by genotypes of oxidative stress-related SNPs (Chahine et al., 2007; Probst-Hensch et al., 2008; Schwartz et al., 2005). It is assumed that inhaled PM induces oxidative stress, an excess of reactive oxygen species, and a release of inflammatory mediators in the lung, which might lead to an imbalance of the autonomic nervous system and hence to a decreased HRV (Brook et al., 2010). Thus, it is hypothesized that people with a reduced oxidative defense due to genetic predispositions might be especially susceptible to PM exposure. In our study, we investigated possible air pollution effect modifications by SNPs involved in changes of cardiac rhythm as reported in GWAS (Newton-Cheh et al., 2007). We hypothesized that people with an altered HRV due to genetic predispositions might also show different reactions to air pollution exposure. We detected the strongest air pollution effects on SDNN in individuals with at least on minor allele of rs333229 but no main effect of this SNP (Supplementary material, Table 1). This SNP is located in the 3′untranslated region of the choline transporter gene (*CHT1*) (Neumann et al., 2005). *CHT*1 encodes the high-affinity choline transporter (ChT), which carries choline into acetylcholine (ACh)-synthesizing neurons. ACh is a neurotransmitter of the sympathetic and parasympathetic system. Therefore, variations in *CHT1* may account for variations in ACh neurotransmission, which might lead to modulation of HR and HRV. Furthermore, it has also been shown that rs333229 might predict intimia-media thickness and plaque occurrence (Neumann et al., 2011). However, we can only speculate that changes in ChT affect the response to air pollution. rs2966762, rs1871841, rs2096767, and rs2745967 modified the air pollution effects as well. Little is known about biological pathways of these SNPs and their influence on the cardiovascular system. rs2096767 is located in the matrix metallopeptidase 13 gene, which is involved in a wide variety of physiological and pathological processes, including normal cell growth, differentiation, and cell regulation (Leeman et al., 2002). Newton-Cheh et al. (2007) observed a significant modulation of the PR-interval by rs2096767 in a GWAS. As all SNPs are located on different chromosomes and are uncorrelated (data not shown) we assume that the unknown underlying biological mechanisms could be different for each SNP. In general, exploring interactions between SNPs and air pollution might help to identify possible biological mechanisms and pathways of air pollution effects on cardiac health.

4.4. Metabolic disorders and susceptibility to air pollution

The risk of developing CAD or suffering from an MI is increased in persons with T2M (Beckman et al., 2002). It has also been shown that insulin resistance might cause a reduction of the autonomic nervous system leading to a reduced HRV in both T2D and IGT groups (Perciaccante et al., 2006). Furthermore, authors reported QTc-prolongation, a marker of ventricular arrhythmias, and a higher risk for emergency department visits and hospital admissions for individuals with T2D compared to individuals without T2D in association with air pollution increases (Baja et al., 2010; Peel et al., 2007; Zanobetti and Schwartz, 2001). Therefore, we assumed that participants with T2D or IGT might be especially susceptible to air pollutants. However, we observed no air pollution effects in individuals with T2D—only in IGT participants. By selecting subjects with IGT, we intended to study the impact of air pollutants in subjects with an enhanced risk for T2D but who were not treated by beta-blockers, statins, or anti-diabetic medications as these medications may modify the effects of particle exposures. Accordingly, a study among people with T2D (Rious et al., 2011) has shown that individuals on insulin were more susceptible to traffic exposure leading to elevated levels of inflammation than those on oral hypoglycemic medication. In our study 71% of participants with T2D took beta-blockers, statins, or oral anti-diabetic medication but only three subjects were on insulin. This may have reduced the possibility to detect ambient air pollution effects on ECG-parameters for individuals with T2D.

People with diabetes are known to have disproportional reactive oxygen species formation (Maritim et al., 2003) and we speculate that this might be also true for individuals with IGT. PM has been hypothesized to cause adverse health effects through the same mechanism. Therefore, diabetes and PM may share common pathways and interact to enhance responsiveness to air pollutants.

4.5. Strengths and limitations

A strength of our study is the ability to analyze intra-individual variation in 1 h-averages of ECG parameters measured repeatedly in up to four ECG recordings with an average duration of 5.6 h. Models were adjusted for long-term time trend and meteorological variables to account for the possibility that the detected associations resulted from meteorological influences or seasonal differences alone. We controlled for circadian variation by design as the repeated ECG recordings started at the same time (± 2 h) for each participant, respectively, and models were adjusted for time of day in case of model fit improvement. A variety of outcome, exposure, and interaction variables have been used in our analyses; thus, some associations may have occurred only by chance. However, we reduced the number of performed tests by selecting SNPs with regression trees in a prior step and we did not include all 139 SNPs as potential air pollution effect modifiers. Furthermore, regression trees are not based on distributional assumptions. No additional tests were carried out in the SNP selection process. Nevertheless, we did not adjust our analyses for multiple testing as our analyses should be regarded as explorative. A limitation of our study is that only one central measurement site was used for the collection of ambient air pollution, which poses a source for exposure misclassification as it assumes homogeneous exposure for the whole study area. Especially ultrafine particles are spatially heterogeneous and depend on distance from the roadway as they are mostly produced by local traffic. However, Cyrys et al. (2008) investigated the temporal and spatial variation of particle number concentrations (PNC) at four background sites in Augsburg and reported high temporal correlations ($r > 0.80$). Therefore, the use of one single ambient monitoring site is a defendable approach for characterizing exposure to ultrafine particles in this specific study area. Moreover, we assume that exposure misclassification by use of air pollution measurements of one central site would probably be non-differential and bias the results toward the null. A further strength of this study is the investigation of air pollution effects in a particularly susceptible subgroup. On the other hand, the results cannot be generalized to the whole population.

5. Conclusion

We observed a reduced HRV, predominantly SDNN, in association with concurrent and delayed increases in 1 h-averages of PM and UFP exposure. These associations were modified by SNPs involved in cardiac rhythm. Therefore, we identified a genetic predisposition potentially making participants with metabolic disorders more susceptible to air pollutants with regard to HRV, a possible precursor of cardiac adverse events. Moreover, as the prevalence of diabetes is increasing worldwide it is important to conduct further investigations in this susceptible population.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2011.10.007.

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