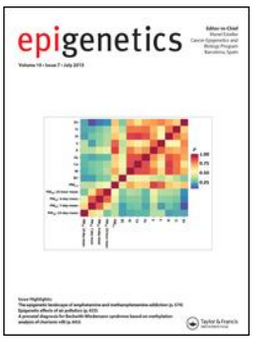

Epigenetics

ISSN: 1559-2294 (Print) 1559-2308 (Online) Journal homepage: http://www.tandfonline.com/loi/kepi20

An epigenome-wide association analysis of cardiac autonomic responses among a population of welders

Jinming Zhang, Zhonghua Liu, Peter E. Umukoro, Jennifer M. Cavallari, Shona C. Fang, Marc G. Weisskopf, Xihong Lin, Murray A. Mittleman & David C. Christiani

To cite this article: Jinming Zhang, Zhonghua Liu, Peter E. Umukoro, Jennifer M. Cavallari, Shona C. Fang, Marc G. Weisskopf, Xihong Lin, Murray A. Mittleman & David C. Christiani (2017) An epigenome-wide association analysis of cardiac autonomic responses among a population of welders, Epigenetics, 12:2, 71-76, DOI: 10.1080/15592294.2016.1270486

To link to this article: https://doi.org/10.1080/15592294.2016.1270486

© 2017 The Author(s). Published with license by Taylor & Francis Group, LLC© Jinming Zhang, Zhonghua Liu, Peter E. Umukoro, Jennifer M. Cavallari, Shona C. Fang, Marc G. Weisskopf, Xihong Lin, Murray A. Mittleman, and David C. Christiani

Accepted author version posted online: 11 Jan 2017.
Published online: 23 Jan 2017.

Submit your article to this journal

Article views: 639

View Crossmark data

Citing articles: 2 View citing articles

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=kepi20

EPIGENETICS
2017, VOL. 12, NO. 2, 71–76
http://dx.doi.org/10.1080/15592294.2016.1270486

Taylor & Francis
Taylor & Francis Group

BRIEF REPORT

OPEN ACCESS

An epigenome-wide association analysis of cardiac autonomic responses among a population of welders

Jinming Zhang[a], Zhonghua Liu[b], Peter E. Umukoro[a], Jennifer M. Cavallari[c], Shona C. Fang[d], Marc G. Weisskopf[a,e], Xihong Lin[b], Murray A. Mittleman[e,f], and David C. Christiani [a,e,g]

[a]Department of Environmental Health, Harvard T. H. Chan School of Public Health, Boston, MA, USA; [b]Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, MA, USA; [c]Department of Community Medicine and Health Care, University of Connecticut Health Center, Farmington, CT, USA; [d]Department of Epidemiology, New England Research Institute, Watertown, NY, USA; [e]Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA, USA; [f]Cardiovascular Epidemiology Research Unit, Beth Israel Deaconess Medical, Center/Harvard Medical School, Boston, MA, USA; [g]Pulmonary and Critical Care Division, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA

ABSTRACT

DNA methylation is one of the potential epigenetic mechanisms associated with various adverse cardiovascular effects; however, its association with cardiac autonomic dysfunction, in particular, is unknown. In the current study, we aimed to identify epigenetic variants associated with alterations in cardiac autonomic responses. Cardiac autonomic responses were measured with two novel markers: acceleration capacity (AC) and deceleration capacity (DC). We examined DNA methylation levels at more than 472,506 CpG probes through the Illumina Infinium HumanMethylation450 BeadChip assay. We conducted separate linear mixed models to examine associations of DNA methylation levels at each CpG with AC and DC. One CpG (cg26829071) located in the *GPR133* gene was negatively associated with DC values after multiple testing corrections through false discovery rate. Our study suggests the potential functional importance of methylation in cardiac autonomic responses. Findings from the current study need to be replicated in future studies in a larger population.

ARTICLE HISTORY
Received 21 September 2016
Revised 10 November 2016
Accepted 2 December 2016

KEYWORDS
Acceleration; deceleration; epigenetics; EWAS; GPR133; heart rate

Introduction

Despite the fact that numerous therapies have been developed in cardiovascular medicine, cardiovascular disease (CVD) remains one of the leading causes of morbidity and mortality worldwide.[1-3] Understanding the biological mechanisms and predictors of CVD may have important implications in prevention and treatment of this public health concern. The cardiac autonomic nervous system consists of two major branches—the sympathetic branch and the parasympathetic branch—and it is essential in modulation of cardiac electrophysiology.[4] Failure of parasympathetic control, which is often measured by conventional heart rate variability (HRV) parameters,[5,6] has been a strong predictor of cardiovascular mortality in both high-risk and low-risk populations.[7,8] Meanwhile, the associations of reduced HRV with various environmental and occupational pollutants, such as particulate matter air pollution with aerodynamic diameter $<2.5\ \mu$m (PM2.5),[9,10] ozone,[11] and heavy metals,[12] have been consistently reported. However, the biological mechanisms by which these pollutants trigger alterations in cardiac autonomic responses remain unclear.

Previous genome-wide association studies (GWAS) have identified several genetic variants associated with cardiac autonomic responses.[13,14] More recent studies suggested that epigenetic regulation, such as DNA methylation, may also play an important role in alterations in cardiac autonomic responses. PM2.5 exposure has been associated with both gene-specific methylation (e.g., iNOS gene),[15,16] and HRV changes in previous air pollution studies. To date, no epigenome-wide association study (EWAS) has been conducted to systematically examine DNA methylation and cardiac autonomic responses. Understanding this relationship may help elucidate the biological pathway of cardiac autonomic responses, which may further have implications for the management and development of therapies for cardiovascular disease.

Welders have higher exposure to welding fumes than other trades involved with welding. Welding fumes often contain a variety of hazards, such as metals, gases, and chemicals, which can cause various cardiovascular disorders.[17-19] We previously observed in several cross-sectional studies that welders had decreased HRV parameters following a 6-hour work shift. Further, exposure to metal-rich welding fumes across the work shift was strongly associated with decreased HRV parameters.[17,19] In this study, we conducted an EWAS to identify epigenetic variants that were associated with exposure-induced cardiac autonomic responses. Two novel markers—acceleration capacity (AC) and deceleration capacity (DC)—were used as indices of cardiac autonomic responses.

CONTACT David C. Christiani dchris@hsph.harvard.edu

Published with license by Taylor & Francis Group, LLC © Jinming Zhang, Zhonghua Liu, Peter E. Umukoro, Jennifer M. Cavallari, Shona C. Fang, Marc G. Weisskopf, Xihong Lin, Murray A. Mittleman, and David C. Christiani.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

Table 1. Demographics of study population (n = 75).

Characteristic	
Male	75 (100)[a]
Current smoker	28 (37.3)[a]
Average age (years)	41.6 ± 12.8[b]
Age range (years)	21.6–71.2
Average BMI (kg/m^2)	28.7 ± 5.3[b]
Acceleration capacity (ms)	−6.42 ± 4.05[b]
Deceleration capacity (ms)	7.64 ± 3.64[b]

[a]n (%)
[b]mean ± SD

Results

Characteristics of the study population are shown in Table 1. The study population was comprised of 75 male welders. The majority of them were Caucasian (93.3%) with an age average of 41.6 years (range: 21.6–71.2). Close to 40% were current smokers, and average BMI was 28.7 kg/m^2. The baseline DC and AC values were 7.64 [Standard deviation (SD) = 3.64] ms and −6.42 (SD = 4.05) ms, respectively. Compared with baseline, the estimated post-work natural killer (NK) cell, monocyte, and granulocyte proportions were significantly higher, whereas post-work CD4$^+$ T cell, CD4$^+$ T cell, and B cell proportions were not significantly different (Table 2).

We examined the associations of methylation levels at 472,506 CpG probes in the whole-genome with AC and DC, while adjusting for age, BMI, smoking status, work-shift, time of sample collection, and cell type composition for whole blood. For DC analyses, only one CpG (cg26829071), located in the *GPR133* gene on chromosome 12 (Fig. 2), reached genome-wide significance using a threshold of false discovery rate (FDR) q-value ≤ 0.1(Table 3). There was a significant negative association between methylation levels at this CpG and DC ($\beta = -0.32$, SE = 0.06; $P = 7.75E\text{-}08$). In addition, we observed suggestive evidence of associations at cg12991522 ($\beta = 0.47$, SE = 0.09; $P = 1.82E\text{-}07$), located in the *PPL* gene on chromosome 16, with DC. For AC analyses, one CpG (cg15273468) was genome-wide significant with FDR q values ≤ 0.1 (Table 4). However, this CpG was not linked with any known functional genes.

The Quantile-Quantile (Q-Q) plots suggested sizable inflation in AC analyses and better calibrated test statistic in DC analyses. To quantify this observation, we calculated the genomic inflation factor (λ) as the median of the observed distribution of the test statistic divided by the expected median. We observed $\lambda = 0.81$ for AC analyses and $\lambda = 1.02$ for DC analyses (Fig. 1). Taken together, the observed associations for DC analyses were unlikely due to population stratification.

Table 2. Estimated cell type composition (%) by time.

Cell type	Post-work[a]	Baseline[a]	*P* value[b]
CD8$^+$T cell	4.27 (4.66)	4.08 (4.59)	0.39
CD4$^+$T cell	13.89 (4.67)	13.73 (4.21)	0.81
NK cell	4.47 (4.61)	2.93 (3.22)	<0.01
B cell	3.68 (2.5)	3.92 (2.35)	0.08
Monocyte	7.64 (2.45)	7.08 (2.5)	0.04
Granulocyte	64.15 (9.23)	66.27 (8.13)	<0.01

[a]Unadjusted mean percentage (SD); n = 208
[b]Comparison between prior and post exposure using linear mixed regression models.

Discussion

In the current study, we aimed to identify epigenetic locations at which the methylation levels were associated with cardiac autonomic changes among a population of welders. There was a significantly negative association between methylation at cg26829071 and DC values. Annotation analysis indicated that this significant CpG was located in the gene body of the *GPR133* gene.

GPR133 encodes an adhesion G-protein-coupled receptor (aGPCR), which is commonly characterized by long extracellular N termini that are composed of a seven transmembrane spanning domain.[20] It is primarily expressed in the central nervous system[21] as well as heart (ventricles, atria, and septal tissues). Bohnekamp et al.[22] have reported a concentration-dependent relationship between GPR133 and intracellular cyclic adenosine 3′,5′-cyclic adenosine monophosphate (cAMP) levels, suggesting that GPR133 may be coupled to the Gs protein, activate adenylate cyclase, and stimulate G protein cascades. In the heart, the activation of adenylate cyclase is associated with various cardiovascular effects including modulation of heart rate. Adenylyl cyclase may activate the production of intracellular cAMP,[23,24] which serves as the second messenger that further binds to protein kinase A and modulates cardiac contractility.[25] Meanwhile, intracellular cAMP is also essential for the generation of action potential in the sinoatrial node.[26] However, further research is needed to replicate previous findings and to explain the role of GRP133 in cardiac autonomic dysfunction.

Both toxicological studies and GWAS have documented associations of several subtypes of GPCRs with adverse cardiovascular effects, such as hyperproliferative vascular malformations (GPR124),[27] myocardial wall thinning (GPR126),[28] stroke (CELSR1),[29] and myocardial infarction (CELSR2).[30] To date, several GWAS studies have been conducted to explore genetic contributions to cardiac autonomic responses. Arking et al.[14] identified the *NOS1AP (CAPON)* gene associated with the electrocardiographic (ECG) QT interval variations among participants from the KORA cohort in Germany. Marroni et al.[13] observed similar findings and they additionally identified the associations of variants in *GPR133* gene with the ECG RR interval alterations. Newton-Cheh et al.[31] examined 70,987 common genetic variants and six conventional heart rate variability (HRV) parameters among 1345 participants from the Framingham Heart Study Original and Offspring cohort and found there was no genomic hit that yielded a genome-wide significance. Our study, however, expands the literature with EWAS and suggests that epigenetic regulation of the *GPR133* gene may also play a role in heart rate modulation.

The role of DNA methylation in regulation of gene-expression may vary depending upon different genomic contexts.[32] DNA methylation in the promoter sequences is known to downregulate gene expression,[33] whereas methylation in the gene body is often positively associated with gene expression.[34] Decreased DC, which reflects impaired cardiac autonomic

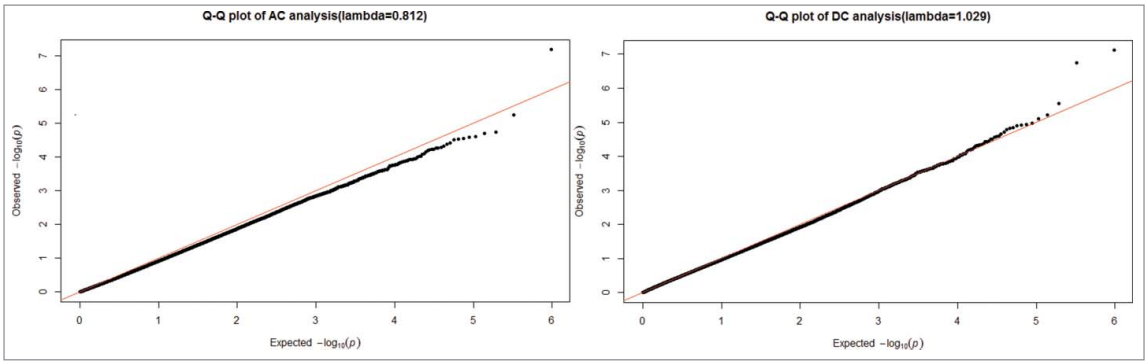

Figure 1. Quantile-Quantile (Q-Q) plot. The observed *P*-values (Y-axis) were plotted against the expected *P*-values under the null hypothesis (X-axis). The red diagonal line denotes the pattern under null hypothesis.

function, has been identified as a strong predictor of cardiovascular mortality among patients after myocardial infarction.[35,36] Our study suggests the important role of DNA methylation in cardiac autonomic dysfunction. However, the underlying biological mechanisms of whether and how methylation at cg26829071 may affect gene expression and induce decreased DC remain to be elucidated.

The second-ranked CpG associated with DC is located in the *PPL* gene (encoding periplakin). The *PPL* gene is a protein-coding gene responsible for keratinocyte intercellular adhesion as well as tissues integrity.[37] Decreased periplakin expression has been associated with urothelial carcinoma of the urinary bladder (UCB),[38] and serum periplakin has been suggested as a biomarker for UCB.[39] However, there was no evidence suggesting a role for periplakin in cardiovascular disease. Its association with cardiac autonomic responses may warrant further research.

To the best of our knowledge, this is the first EWAS investigating epigenetic variants associated with cardiac autonomic responses. GPCRs are one of most studied receptor families that are used as pharmacological targets.[40] In the heart, the adrenergic GPCR signaling pathways are within the major targets of pharmaceuticals for the treatment of cardiovascular disease. For example, beta-blockers have been extensively used for the management of arrhythmia, hypertension, and chronic heart failure.[41] Our study indicated a potential role of *GPR133* in cardiac autonomic dysfunction, primarily through affecting the deceleration capacity of heart rate. Future studies may investigate the association of the *GPR133* gene with various cardiovascular diseases, such as abnormal heart rhythms and heart failure.

Our study is limited by the relatively small sample size and lack of independent replication. Therefore, our findings need to be confirmed in future studies in a larger population. In addition, previous studies have reported strong associations of acute and chronic PM2.5 exposure with DNA methylation in several genes as well as cardiac autonomic responses. Both short-term and long-term particulate matter exposure from welding fumes were significantly associated with AC and DC as well as conventional HRV parameters changes among this welder cohort.[17,42] PM2.5 exposure has also been negatively associated with gene-specific *(iNOS)* methylation in a population of boilermaker construction workers as well as elderly men from the VA Normative Aging Study.[16] Hence, we cannot preclude the potential confounding effects of PM2.5 due to lack of data in this study. Meanwhile, there might be other potential confounders in the occupational settings, such as heavy metals and psychological factors, in addition to PM2.5.

In conclusion, results of our study indicate that methylation at the *GPR133* gene may play a role in cardiac autonomic dysfunction. However, we cannot preclude chance or bias due to lack of replication, small sample size, and potential confounding by pollutants exposure. Hence, our preliminary findings need to be confirmed in future research.

Materials and methods

Study population

The Harvard Boilermakers Study is a prospective cohort study conducted among a population of welders from a local boiler-

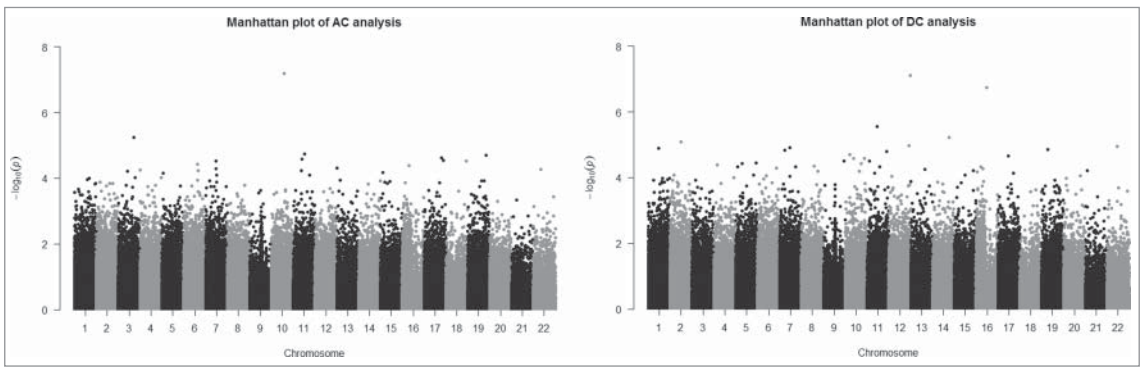

Figure 2. Manhattan Plots for AC and DC analysis. The Manhattan plot denoting the *P*-values for the association of DNA methylation levels at autosomal sites (X-axis).

Table 3. List of top-ranking CpGs associated with DC.

Name	Chromosome	Strand	Gene name	Location	β	SE	*P* value	FDR q value
cg26829071	12	F	GPR133	Body	−0.32	0.06	7.75E-08	0.04
cg12991522	16	F	PPL	1stExon	0.47	0.09	1.82E-07	0.04
cg12071328	11	F	NELL1	Body	0.39	0.08	2.81E-06	0.44
cg20928238	14	R			−0.24	0.05	6.11E-06	0.64
cg01520463	2	F	SDC1	Body	0.18	0.04	8.03E-06	0.64
cg25048985	12	F			−0.29	0.06	1.05E-05	0.64
cg12454595	22	R	FAM19A5	Body	0.68	0.15	1.14E-05	0.64
cg12479444	7	F			0.62	0.13	1.21E-05	0.64
cg12044338	1	F	AQP10	TSS1500	0.41	0.09	1.27E-05	0.64
cg06716283	19	F	RELB	TSS1500	0.39	0.09	1.42E-05	0.64

maker union in Quincy, MA. These welders primarily assemble or weld boilers in large power plants. The study population included 75 welders recruited from 6 sampling occasions in January 2003, January 2004, June 2010, January 2011, June 2011, and June 2012. Workers were allowed to participate multiple times within the 6 sampling occasions. The inclusion criteria were: a) male welders $\geq$ 18 years of age; b) unionized welders including both apprentice and journeyman; c) contributed blood and ECG samples at least once. The exclusion criteria were self-reported physician diagnosed cardiovascular disease prior participation.

During each sampling occasion, participants were monitored at a union welding school on an approximate 6-hour workday. Their major occupational activities included welding, grinding, and cutting tasks. We collected urine, blood, and resting ECG recordings at both prior- (baseline) and post-work. We also collected self-administrated questionnaires including age, height, weight, current smoking status and/or smoking history, medical history, and medication use during the previous six months from each participant at baseline, with this information being updated at each sampling occasion. The Harvard T. H. Chan School of Public Health Institutional Review Board reviewed and approved the study protocol and we obtained a written informed consent from each participant at each sampling occasion.

Electrocardiographic recording and sample analysis

During each sampling occasion, participants were fitted with a 7-lead ambulatory ECG Holter monitor. We collected 12-minute resting ECG recordings from each participant at both prior- and post-work. During the 12-minute resting period, participants were asked to remain seated and quiet; walking, talking, or eating was not allowed. The ECGs samples were then sent to the Cardiovascular Epidemiology Research Unit of Beth Israel Deaconess Medical Center (Boston, Massachusetts, USA), where trained technicians blinded to exposure status processed and analyzed these samples. The methods of processing ECGs samples for AC and DC analysis have been discussed in our previous study.[17] Briefly, to compute DC values, RR intervals longer than the immediate preceding interval were defined as anchors; to compute AC values, RR intervals shorter than the immediate preceding interval were defined as anchors. Segments of interval data that had the same size around these anchors were identified and aligned at those anchors. Upon alignment of all segments, RR intervals at all defined anchors (X0), immediate preceding (X1) and following the anchors (X-1) were averaged separately. The quantities of AC or DC were obtained by computing the difference between the sum of X0 and X1 and the sum of (X-1) and (X-2).[35] For ECG sample analysis, the first two minutes of the ECG recordings were discarded to allow for acclimation. AC and DC quantities were summarized every 5 minutes through the PRSA method, as described by Bauer et al.[35] A total of 208 5-minute AC and DC values were obtained and repeated measurements of AC and DC values were modeled separately for each subject.

DNA methylation profiling and data quality control

We collected whole blood samples (n = 208) from each participant at both prior- and post-work through venous phlebotomy in EDTA tubes. Plasma was extracted and blood pellets were bisulfite-converted. DNA methylation levels of the entire genome that covers more than 480,000 CG dinucleotide (CpG) probes from both pre- and post-shift blood samples were determined through Infinium HumanMethylation450 BeadChip assay (Illumina, Inc.) following the Infinium HD Methylation Assay protocol guide. The BeadChips were scanned using the Illumina iScan and raw data was imported into GenomeStudio where image intensities were extracted. A methylation score

Table 4. List of top-ranking CpGs associated with AC.

Name	Chromosome	Strand	Gene name	Location	β	SE	*P* value	FDR q value
cg15273468	10	R			−0.31	0.05	6.60E-08	0.03
cg19458608	3	R	LOC729375	TSS1500	−0.92	0.19	5.67E-06	1.00
cg14004557	11	F			−0.28	0.06	1.81E-05	1.00
cg26905268	19	R	KANK2	Body	−0.54	0.12	2.00E-05	1.00
cg05434952	17	R	CCDC137	1stExon	−0.95	0.22	2.45E-05	1.00
cg10414946	11	F	MS4A2	Body	−0.42	0.10	2.64E-05	1.00
cg02069944	17	R	AZI1	Body	1.28	0.29	2.83E-05	1.00
cg27445005	18	R			0.49	0.11	2.98E-05	1.00
cg11945929	7	F			−0.15	0.03	3.05E-05	1.00
cg24078451	6	F	PSMB8	1stExon	−0.36	0.08	3.83E-05	1.00

(β value) was quantified on a scale from 0 to 1 to represent the percentage of methylated signal at each CpG, such that a value of 0 represents fully unmethylated signal and a value of 1 represents fully methylated signal.

Following background subtraction and dye bias adjustment, both sample-level and probe-level quality control procedures were performed. Samples with detection P-value > 0.05 in more than 5% probes were excluded; probes with detection P-value > 0.05 in more than 5% samples or probes with very low variation (CV $< 5\%$) were omitted. Sex chromosomes and single nucleotide polymorphism-associated probes were also excluded. In addition, Houseman's algorithm[43] in minfi[44] was applied to estimate the cell type composition in blood samples. Beta-mixture quantile normalization[45] was conducted for probe design bias correction. The β values were normalized with functional normalization and batch effect adjustment was performed with ComBat.[46] In total, there were 472,506 CpG probes included in the final association analyses.

Statistical analysis

In the current study, we compared the post-work blood cell type composition with baseline composition through linear mixed effects models. We fitted separate linear mixed effects models with random intercepts to examine whether DNA methylation at each CpG was associated with AC and DC. A number of covariates including age (continuous in years), body mass index (BMI; continuous weight in kilograms divided by height squared in meters), current smoking status (non-smoker, former smoker, or current smoker), time (baseline or post-work) and cell type composition were adjusted for in all models as they have been suggested as potential confounders. In addition, all models were adjusted for the variable "time in day," which reflects the time when blood and ECG samples were collected, to account for the potential circadian variations of AC and DC.

To correct for multiple comparisons, we computed the false discovery rate (FDR) adjusted P- value (q value). We considered an FDR q value ≤ 0.1 as statistically significant.

We fitted the QQ plot to visualize the expected distribution of test statistics of the association analyses across the CpG probes (X-axis) vs. the observed values (Y-axis). We also calculated the genomic inflation factor (lambda) to assess the bulk inflation and false positive rate.[47] All analyses were performed with R 3.2.2 (R Core Team 2015).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

The current research was supported by NIEHS ES009860 (to DC), ES000002 (to DC), T32 ES 07069 (to DC), Harvard Education and Research Center (ERC -T42OH008416 to DC), an American Heart Association Scientist Development award to S Fang.

ORCID

David C. Christiani http://orcid.org/0000-0002-0301-0242

References

1. Fuster V. Global burden of cardiovascular disease: time to implement feasible strategies and to monitor results. J Am Coll Cardiol 2014; 64:520-2; PMID:25082587; http://dx.doi.org/10.1016/j.jacc.2014.06.1151
2. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. Circulation 2001; 104:2746-53; PMID:11723030; http://dx.doi.org/10.1161/hc4601.099487
3. Mensah GA, Brown DW. An overview of cardiovascular disease burden in the United States. Health Aff (Millwood) 2007; 26:38-48; PMID:17211012; http://dx.doi.org/10.1377/hlthaff.26.1.38
4. Shen MJ, Zipes DP. Role of the autonomic nervous system in modulating cardiac arrhythmias. Circ Res 2014; 114:1004-21; PMID:24625726; http://dx.doi.org/10.1161/CIRCRESAHA.113.302549
5. Heart rate variability for risk stratification of life-threatening arrhythmias. American College of Cardiology Cardiovascular Technology Assessment Committee. J Am Coll Cardiol 1993; 22:948-50; PMID:8354837; http://dx.doi.org/10.1016/0735-1097(93)90217-O
6. 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 1996; 93:1043-65; PMID:8598068; http://doi.org/10.1161/01.CIR.93.5.1043
7. Colhoun HM, Francis DP, Rubens MB, Underwood SR, Fuller JH. The association of heart-rate variability with cardiovascular risk factors and coronary artery calcification: a study in type 1 diabetic patients and the general population. Diabetes Care 2001; 24:1108-14; PMID:11375379; http://dx.doi.org/10.2337/diacare.24.6.1108
8. Tsuji H, Venditti FJ, Jr., Manders ES, Evans JC, Larson MG, Feldman CL, Levy D. Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. Circulation 1994; 90:878-83; PMID:8044959; http://dx.doi.org/10.1161/01.CIR.90.2.878
9. Gold DR, Litonjua A, Schwartz J, Lovett E, Larson A, Nearing B, Allen G, Verrier M, Cherry R, Verrier R. Ambient pollution and heart rate variability. Circulation 2000; 101:1267-73; PMID:10725286; http://dx.doi.org/10.1161/01.CIR.101.11.1267
10. de Hartog JJ, Lanki T, Timonen KL, Hoek G, Janssen NA, Ibald-Mulli A, Peters A, Heinrich J, Tarkiainen TH, van Grieken R, et al. Associations between PM2.5 and heart rate variability are modified by particle composition and beta-blocker use in patients with coronary heart disease. Environ Health Perspect 2009; 117:105-11; PMID:19165395; http://dx.doi.org/10.1289/ehp.11062
11. Jia X, Song X, Shima M, Tamura K, Deng F, Guo X. Acute effect of ambient ozone on heart rate variability in healthy elderly subjects. J Expo Sci Environ Epidemiol 2011; 21:541-7; PMID:21522190; http://dx.doi.org/10.1038/jes.2011.18
12. Feng W, He X, Chen M, Deng S, Qiu G, Li X, Liu C, Li J, Deng Q, Huang S, et al. Urinary metals and heart rate variability: a cross-sectional study of urban adults in Wuhan, China. Environ Health Perspect 2015; 123:217-22; PMID:25356836; http://dx.doi.org/10.1289/ehp.123-A217
13. Marroni F, Pfeufer A, Aulchenko YS, Franklin CS, Isaacs A, Pichler I, Wild SH, Oostra BA, Wright AF, Campbell H, et al. A genome-wide association scan of RR and QT interval duration in 3 European genetically isolated populations: the EUROSPAN project. Circ Cardiovasc Genet 2009; 2:322-8; PMID:20031603; http://dx.doi.org/10.1161/CIRCGENETICS.108.833806
14. Arking DE, Pfeufer A, Post W, Kao WH, Newton-Cheh C, Ikeda M, West K, Kashuk C, Akyol M, Perz S, et al. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. Nat Genet 2006; 38:644-51; PMID:16648850; http://dx.doi.org/10.1038/ng1790
15. Kile ML, Fang S, Baccarelli AA, Tarantini L, Cavallari J, Christiani DC. A panel study of occupational exposure to fine particulate matter and changes in DNA methylation over a single workday and years

worked in boilermaker welders. Environ Health 2013; 12:47; PMID:23758843; http://dx.doi.org/10.1186/1476-069X-12-47
16. Madrigano J, Baccarelli A, Mittleman MA, Sparrow D, Spiro A, 3rd, Vokonas PS, Cantone L, Kubzansky L, Schwartz J. Air pollution and DNA methylation: interaction by psychological factors in the VA Normative Aging Study. Am J Epidemiol 2012; 176:224-32; PMID:22798479; http://dx.doi.org/10.1093/aje/kwr523
17. Umukoro PE, Cavallari JM, Fang SC, Lu C, Lin X, Mittleman MA, Christiani DC. Short-term metal particulate exposures decrease cardiac acceleration and deceleration capacities in welders: a repeated-measures panel study. Occup Environ Med 2016; 73:91-6; PMID:26644456; http://dx.doi.org/10.1136/oemed-2015-103052
18. Cavallari JM, Eisen EA, Fang SC, Schwartz J, Hauser R, Herrick RF, Christiani DC. PM2.5 metal exposures and nocturnal heart rate variability: a panel study of boilermaker construction workers. Environ Health 2008; 7:36; PMID:18613971; http://dx.doi.org/10.1186/1476-069X-7-36
19. Fan T, Fang SC, Cavallari JM, Barnett IJ, Wang Z, Su L, Byun HM, Lin X, Baccarelli AA, Christiani DC. Heart rate variability and DNA methylation levels are altered after short-term metal fume exposure among occupational welders: a repeated-measures panel study. BMC Public Health 2014; 14:1279; PMID:25512264; http://dx.doi.org/10.1186/1471-2458-14-1279
20. Bjarnadottir TK, Fredriksson R, Hoglund PJ, Gloriam DE, Lagerstrom MC, Schioth HB. The human and mouse repertoire of the adhesion family of G-protein-coupled receptors. Genomics 2004; 84:23-33; PMID:15203201; http://dx.doi.org/10.1016/j.ygeno.2003.12.004
21. Vanti WB, Nguyen T, Cheng R, Lynch KR, George SR, O'Dowd BF. Novel human G-protein-coupled receptors. Biochem Biophys Res Commun 2003; 305:67-71; PMID:12732197; http://dx.doi.org/10.1016/S0006-291X(03)00709-5
22. Bohnekamp J, Schoneberg T. Cell adhesion receptor GPR133 couples to Gs protein. J Biol Chem 2011; 286:41912-6; PMID:22025619; http://dx.doi.org/10.1074/jbc.C111.265934
23. Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning receptors and heart function. Nature 2002; 415:206-12; PMID:11805844; http://dx.doi.org/10.1038/415206a
24. Xiang Y, Kobilka BK. Myocyte adrenoceptor signaling pathways. Science 2003; 300:1530-2; PMID:12791980; http://dx.doi.org/10.1126/science.1079206
25. Koch WJ, Rockman HA, Samama P, Hamilton RA, Bond RA, Milano CA, Lefkowitz RJ. Cardiac function in mice overexpressing the beta-adrenergic receptor kinase or a beta ARK inhibitor. Science 1995; 268:1350-3; PMID:7761854; http://dx.doi.org/10.1126/science.7761854
26. Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol 2003; 65:453-80; PMID:12471170; http://dx.doi.org/10.1146/annurev.physiol.65.092101.142734
27. Kuhnert F, Mancuso MR, Shamloo A, Wang HT, Choksi V, Florek M, Su H, Fruttiger M, Young WL, Heilshorn SC, et al. Essential regulation of CNS angiogenesis by the orphan G protein-coupled receptor GPR124. Science 2010; 330:985-9; PMID:21071672; http://dx.doi.org/10.1126/science.1196554
28. Patra C, van Amerongen MJ, Ghosh S, Ricciardi F, Sajjad A, Novoyatleva T, Mogha A, Monk KR, Muhlfeld C, Engel FB. Organ-specific function of adhesion G protein-coupled receptor GPR126 is domain-dependent. Proc Natl Acad Sci U S A 2013; 110:16898-903; PMID:24082093; http://dx.doi.org/10.1073/pnas.1304837110
29. Gouveia LO, Sobral J, Vicente AM, Ferro JM, Oliveira SA. Replication of the CELSR1 association with ischemic stroke in a Portuguese case-control cohort. Atherosclerosis 2011; 217:260-2; PMID:21511255; http://dx.doi.org/10.1016/j.atherosclerosis.2011.03.022
30. Qi L, Ma J, Qi Q, Hartiala J, Allayee H, Campos H. Genetic risk score and risk of myocardial infarction in Hispanics. Circulation 2011; 123:374-80; PMID:21242481; http://dx.doi.org/10.1161/CIRCULATIONAHA.110.976613
31. Newton-Cheh C, Guo CY, Wang TJ, O'Donnell C J, Levy D, Larson MG. Genome-wide association study of electrocardiographic and heart rate variability traits: the Framingham Heart Study. BMC Med Genet 2007; 8(Suppl 1):S7; PMID:17903306; http://dx.doi.org/10.1186/1471-2350-8-S1-S7
32. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 2012; 13:484-92; PMID:22641018; http://dx.doi.org/10.1038/nrg3230
33. Payer B, Lee JT. X chromosome dosage compensation: how mammals keep the balance. Annu Rev Genet 2008; 42:733-72; PMID:18729722; http://dx.doi.org/10.1146/annurev.genet.42.110807.091711
34. Jones PA. The DNA methylation paradox. Trends Genet 1999; 15:34-7; PMID:10087932; http://dx.doi.org/10.1016/S0168-9525(98)01636-9
35. Bauer A, Kantelhardt JW, Barthel P, Schneider R, Makikallio T, Ulm K, Hnatkova K, Schomig A, Huikuri H, Bunde A, et al. Deceleration capacity of heart rate as a predictor of mortality after myocardial infarction: cohort study. Lancet 2006; 367:1674-81; PMID:16714188; http://dx.doi.org/10.1016/S0140-6736(06)68735-7
36. Kantelhardt JW, Bauer A, Schumann AY, Barthel P, Schneider R, Malik M, Schmidt G. Phase-rectified signal averaging for the detection of quasi-periodicities and the prediction of cardiovascular risk. Chaos 2007; 17:015112; PMID:17411269; http://dx.doi.org/10.1063/1.2430636
37. Ruhrberg C, Hajibagheri MA, Parry DA, Watt FM. Periplakin, a novel component of cornified envelopes and desmosomes that belongs to the plakin family and forms complexes with envoplakin. J Cell Biol 1997; 139:1835-49; PMID:9412476; http://dx.doi.org/10.1083/jcb.139.7.1835
38. Matsumoto K, Ikeda M, Sato Y, Kuruma H, Kamata Y, Nishimori T, Tomonaga T, Nomura F, Egawa S, Iwamura M. Loss of periplakin expression is associated with pathological stage and cancer-specific survival in patients with urothelial carcinoma of the urinary bladder. Biomed Res 2014; 35:201-6; PMID:24942859; http://dx.doi.org/10.2220/biomedres.35.201
39. Matsumoto K, Ikeda M, Matsumoto T, Nagashio R, Nishimori T, Tomonaga T, Nomura F, Sato Y, Kitasato H, Iwamura M. Serum periplakin as a potential biomarker for urothelial carcinoma of the urinary bladder. Asian Pac J Cancer Prev 2014; 15:9927-31; PMID:25520130; http://dx.doi.org/10.7314/APJCP.2014.15.22.9927
40. Ghanemi A. Targeting G protein coupled receptor-related pathways as emerging molecular therapies. Saudi Pharm J 2015; 23:115-29; PMID:25972730; http://dx.doi.org/10.1016/j.jsps.2013.07.007
41. Exner DV, Reiffel JA, Epstein AE, Ledingham R, Reiter MJ, Yao Q, Duff HJ, Follmann D, Schron E, Greene HL, et al. Beta-blocker use and survival in patients with ventricular fibrillation or symptomatic ventricular tachycardia: the Antiarrhythmics Versus Implantable Defibrillators (AVID) trial. J Am Coll Cardiol 1999; 34:325-33; PMID:10440140; http://dx.doi.org/10.1016/S0735-1097(99)00234-X
42. Umukoro PE, Fan T, Zhang J, Cavallari JM, Fang SC, Lu C, Lin X, Mittleman MA, Schmidt G, Christiani DC. Long-Term Metal PM2.5 Exposures Decrease Cardiac Acceleration and Deceleration Capacities in Welders. J Occup Environ Med 2016; 58:227-31; PMID:26949871; http://dx.doi.org/10.1097/JOM.0000000000000661
43. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, Wiencke JK, Kelsey KT. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics 2012; 13:86; PMID:22568884; http://dx.doi.org/10.1186/1471-2105-13-86
44. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, Irizarry RA. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 2014; 30:1363-9; PMID:24478339; http://dx.doi.org/10.1093/bioinformatics/btu049
45. Teschendorff AE, Marabita F, Lechner M, Bartlett T, Tegner J, Gomez-Cabrero D, Beck S. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. Bioinformatics 2013; 29:189-96; PMID:23175756; http://dx.doi.org/10.1093/bioinformatics/bts680
46. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics 2007; 8:118-27; PMID:16632515; http://dx.doi.org/10.1093/biostatistics/kxj037
47. Devlin B, Roeder K. Genomic control for association studies. Biometrics 1999; 55:997-1004; PMID:11315092; http://dx.doi.org/10.1111/j.0006-341X.1999.00997.x