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An environmental wide association study (EWAS) approach to the metabolic syndrome ☆

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ABSTRACT

Background: Environmental contaminants have previously been linked to components of the Metabolic Syndrome (MetS). However, exposure to environmental contaminants is in part determined by various lifestyle factors.
Objective: Using an "Environmental Wide Association Study" (ELWAS) integrating environmental contaminants and lifestyle factors, we aimed to evaluate a possible additive role of both contaminants and lifestyle factors regarding MetS.
Methods: 1016 subjects aged 70 years were investigated in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. 43 environmental contaminants were measured in the circulation. Dietary records were used to evaluate 21 nutrients and the proportions of 13 fatty acids were determined in serum cholesterol esters to further quantify fat quality intake. Adding 5 other important lifestyle factors yielded together 76 environmental and lifestyle factors. MetS was defined by the NCEP/ATPIII-criteria.
Results: 23% had MetS. Using cross-validation within the sample, fourteen environmental contaminants or lifestyle factors consistently showed a false discovery rate <0.05. When the major variables entered a multiple model, only *p*, *p*′-DDE levels (positive), PCB209 (inverse) and exercise habits (inverse) were together with a fatty acid pattern, with high levels of palmitic acid and oleic acid and low levels of linoleic acid, related to MetS ($p<0.002$ for all variables).
Conclusion: Using a cross-sectional EWAS approach, certain environmental contaminants and lifestyle factors were found to be associated with prevalent metabolic syndrome in an additive fashion in an elderly population.

1. Introduction

A number of environmental contaminants of different kinds, e.g. persistent organic pollutants (POPs), plastic associated chemicals (PACs) and heavy metals, have been linked to different risk factors for cardiovascular disease (CVD) such as obesity, diabetes, hypertension and dyslipidemia (Hatch et al., 2010; Lee et al., 2010, 2011; Lind et al., 2012a,b, 2013; Olsen et al., 2012a; Rignell-Hydbom et al., 2010; Ronn et al., 2011; Roos et al., in press; Ruzzin et al., 2010; Sergeev and Carpenter, 2010; Stahlhut et al., 2007). These cardiometabolic risk factors are commonly aggregating in the same individual, a high-risk state known as The Metabolic syndrome (MetS) (Alberti and Zimmet, 1998; Anonymous, 2001; Lind et al., 1988; Reaven, 1988). Some recent studies have shown POPs to be linked to this syndrome (Lee et al., 2007; Uemura et al., 2009).

However, an important confounder regarding the relationship between environmental pollutants and MetS is the lifestyle of the individual. Several lifestyle factors, such as diet, smoking, social group, and occupation, are linked to the exposure to different environmental pollutants. Thus, a comprehensive evaluation of the links between environmental contaminants should account for lifestyle factors as well.

Recently, Chirag J. Patel and co-workers presented an attractive way to present associations between multiple measured environmental contaminants and diabetes using data from the National Health and Nutrition Examination Survey (NHANES) study (Patel et al., 2010). They used a format commonly used in genetic studies, Genetic Wide Association Study (GWAS), and then applied this format to environmental factors, Environmental Wide Association Study (EWAS). These authors have later applied this format also in other EWAS studies (Patel et al., 2012; Tzoulaki et al., 2012). An advantage of this format is the systematic use of all environmental factors evaluated, since this approach will limit the

Abbreviations: BDE, bromated diphenyl ether; CVD, cardiovascular disease; *p*,*p*′-DDE, 1,1-dichloro-2,2-bis(4-dichlordiphenyl) ethylene; a, metabolite to DDT; EWAS, Environmental Wide Association Study; GWAS, Genetic Wide Association Study; HCB, hexachlorobenzene; OCDD, octachlorodibenzo-p-dioxin; MetS, Metabolic Syndrome; NHANES, National Health and Nutrition Examination Survey; PACs, plastic associated chemicals; PCBs, polychlorinated biphenyls; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; POPs, persistent organic pollutants; TCDD, tetrachlorodibenzo-p-dioxin; TEQ, toxic equivalents; TNC, trans-nonachlordane.

☆ Disclosures: None.

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number of false positive findings induced by selective reporting of significant results, as previously been discussed by Ioannidis and others (Blair et al., 2009; Boffetta et al., 2008; Fallin and Kao, 2011; Ioannidis, 2005, 2008; Ioannidis et al., 2009; Young, 2010).

In the original ELWAS format, Patel and co-workers mainly used lifestyle factors, such as diet, exercise habits, socio-economic status, smoking etc., as confounders in the analysis. Since many of these lifestyle factors could well be an integrated part of the load of environmental factors, we propose an extension of the EWAS concept to also include major lifestyle factors in the main analysis. We used cross-sectional data from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (Lind et al., 2005) in which we in a systematic way used data on a large set of environmental contaminants, as well as dietary records, measurement of fatty acids in cholesterol esters to further explore the quality of fat intake in more detail in addition to history of major lifestyle factors. We hypothesized that environmental contaminants and lifestyle factors are related to prevalent MetS in an additive fashion.

2. Material and methods

2.1. Subjects

Eligible subjects were all aged 70 and lived in the City of Uppsala, Sweden, a City with 170,000 inhabitants. The subjects were randomly chosen from the register of community living. A total of 1016 subjects participated, giving a participation rate of 50.1%. Only one immigrant (from China) was included in the sample.

The study was approved by the Ethics Committee of Uppsala University, and all the participants gave their informed consent prior to the study.

All subjects were investigated in the morning after an over-night fast. No medication or smoking was allowed after midnight. The participants were asked to answer a questionnaire about their medical history, smoking habits and regular medication.

Blood pressure was measured by a calibrated mercury sphygmomanometer in the non-cannulated arm to the nearest mm Hg after at least 30 min of rest and the average of three recordings was used. Lipid variables and fasting blood glucose were measured by standard laboratory techniques.

All environmental and lifestyle variables measured in the PIVUS study with <5% missing values were included in the present evaluation.

Basic characteristics are given in Table 1.

Approximately 10% of the cohort reported a history of coronary heart disease, 4% reported stroke, and 9% reported diabetes mellitus. Almost half the cohort reported some sort of cardiovascular medication (45%), with antihypertensive medication being the most prevalent (32%). Fifteen percent reported the use of statins, while insulin and oral antiglycemic drugs were reported in 2 and 6%, respectively — see reference (Lind et al., 2005) for details.

2.2. Chemical analysis

2.2.1. Metals

All 11 metal elements in this study were determined in whole blood. The analysis was performed using inductively coupled plasma-sector field mass spectrometry, ICP-SFMS, after microwave-assisted digestion with nitric acid (Rodushkin and Axelsson, 2003) according to a method accredited for 10 of the 11 metal elements tested, with Al being unaccredited. Further details can be found elsewhere (Olsen et al., 2012a).

2.2.2. Bisphenol A and phthalates

Human serum was analyzed for levels of bisphenol A (BPA) and ten phthalate metabolites (mono-[2-ethyl-5-hydroxyhexyl] phthalate [MEHHP]; mono [2-ethyl-5-oxohexyl] phthalate [MEOHP]; mono-[2-ethylhexyl] phthalate [MEHP]; monobenzyl phthalate [MBZP]; monocyclohexyl phthalate [MCHP]; monoethyl phthalate [MEP]; monoisobutyl phthalate [MIBP]; monoisononyl phthalate [MINP]; monomethyl phthalate [MMP]; and mono-n-octyl phthalate [MOP]) at ALS Canada following the general procedures presented by the Centers for Disease Control and Prevention Detectable levels were found in almost all subjects for four phthalate metabolites, MEHP, MEP MIBP and MMP. Further details can be found elsewhere (Olsen et al., 2012b).

2.2.3. Persistent organic pollutants POPs

POPs were measured in stored plasma samples collected at baseline. Analyses of POPs were performed using a Micromass Autospec Ultima (Waters, Milford, MA, USA) high resolution chromatography coupled to high resolution mass spectrometry (HRGC/HRMS) system based on the method by Sandau et al. (Sandau et al., 2003) with some modifications. All details on POP analyses including quality control are provided in elsewhere (Salihovic et al., 2012). A total of 23 POPs were measured: 16 polychlorinated biphenyl (PCB) congeners, 5 organochlorine (OC) pesticides, 1 octachlorodibenzo-*p*-dioxin (OCDD), and 1 polybrominated diphenyl ether (PBDE) congener. Among the 23 POPs measured, 2 OC pesticides (*trans*-chlordane and *cis*-chlordane) with detection rates <10% were not included in the final statistical analyses; the minimum detection rate was 72%, and was observed for BDE47. Plasma concentrations were lipid adjusted and are expressed as ng/g lipid.

2.3. Biomarkers of dietary fat quality

Fatty acid composition was measured in serum as an objective marker of the average dietary fatty acid intake during the preceding weeks (Boberg et al., 1985; Ohrvall et al., 1996). Serum was extracted with a hexane-isopropanol solution and cholesterol esters were separated from the extract by thin-layer chromatography before interesterification with acidic methanol was performed. Free cholesterol that had been liberated in the reaction was removed by aluminum oxide to avoid contamination of the column. The percentage composition of methylated fatty acids from 14:0 to 22:6 was determined by gas chromatography (25-m NB-351 silica capillary column) with a flame ionization detector and helium as carrier gas. The CV varied between 0.2% and 5.0% in successive gas chromatography runs. The relative amount of fatty acids is expressed as the percentage of the total amount of fatty acids.

2.4. Definition of the metabolic syndrome

MetS was defined by the NECP/ATP III criteria (2001). Three of the following five criteria should be fulfilled: Blood pressure >130/85 mm Hg or antihypertensive treatment, fasting blood glucose >5.6 mmol/l, serum triglycerides >1.7 mmol/l, waist circumference >102 cm in men and >88 cm in women, HDL-cholesterol <1.0 mmol/l in men and <1.3 in women.

2.5. Definition of lifestyle factors

Educational level was divided into three groups: <9 years, 9–12 years and >12 years of education. Exercise habits were divided into four groups: <2 times light exercise (no sweat) per week, ≥2 times lightexercise per week, 1–2 times heavy exercise (sweat) per week, >2 times heavy exercise (sweat) per week. The dietary intakes of total calories, macronutrients, alcohol and intake of vitamin and trace elements were assessed by use of 7-day food diary and computer software. The subjects were told to give the number of close friends and relatives as a measure of their social network. They were also asked how many hours they usually slept during the night and if they were current smokers or not.