Environment International 119 (2018) 527–535

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Iron-processing genotypes, nutrient intakes, and cadmium levels in the Normative Aging Study: Evidence of sensitive subpopulations in cadmium risk assessment

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ARTICLE INFO

Handling Editor: Lesa Aylward

Keywords:
Cadmium
HFE
Hemochromatosis
Transferrin
Risk assessment
Iron
Nutrients
Metals
Absorption
Gene-environment interaction

ABSTRACT

Background: Because iron and cadmium share common transport mechanisms, iron-processing protein variants such as HFE C282Y, HFE H63D, and Transferrin P570S may influence cadmium metabolism. Our aim was to evaluate associations between common HFE and Transferrin polymorphisms and toenail cadmium levels among older men.

Methods: In a longitudinal cohort of men age 51–97, the Normative Aging Study (NAS), we evaluated toenail cadmium concentrations and missense single nucleotide polymorphisms (SNPs) in the HFE and Transferrin genes. We fit age-adjusted models to estimate associations between genotypes and toenail cadmium concentrations. We then considered potential interactions with smoking status, hemoglobin, and nutritional intakes known to modulate cadmium absorption. For the significant interactions, we also evaluated genotype specific effect estimates.

Results: HFE and Transferrin genotypes were not associated with toenail cadmium concentrations in the main effect analyses, but there were significant interactions between HFE H63D and hemoglobin ($p_{interaction}$ = 0.021), as well as HFE H63D and vitamin C intake ($p_{interaction}$ = 0.048). Genotype specific effect estimates suggested: 1) an inverse relationship between hemoglobin and cadmium levels among HFE H63D homozygotes, and 2) an inverse relationship between vitamin C intake and cadmium levels that strengthens with the number of HFE H63D variant alleles a subject carries.

Conclusions: These findings suggest that sensitive subpopulations defined by diet, hemoglobin level, and genotype may absorb more cadmium from their environment and thus should be considered in cadmium risk analyses. These findings are particularly relevant given the high prevalence of the H63D variant worldwide.

1. Background

Iron, an important nutrient, and cadmium, a toxic contaminant, are both metals with divalent cation forms that are found in food. (ATSDR, 2008; Mackenzie and Garrick, 2005; Schumann et al., 2007) Low body iron stores, and insufficient dietary intake of several other nutrients are known to increase the absorption of cadmium from foods (Fox et al., 1980; Andersen et al., 2004; Flanagan et al., 1978), and evidence described below suggests that iron-processing gene variants might also enhance intestinal cadmium uptake. Therefore, these factors may be risk factors for cadmium mediated diseases such as renal failure, osteoporosis (ATSDR, 2008), and possibly neurodevelopmental/

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https://doi.org/10.1016/j.envint.2018.06.040
Received 27 February 2018; Received in revised form 7 June 2018; Accepted 30 June 2018

neurocognitive dysfunctions (Pihl and Parkes, 1977; Capel et al., 1981; Thatcher et al., 1982; Ciesielski et al., 2012; Viaene et al., 2000; Ciesielski et al., 2013).

Polymorphisms in the HFE gene are present in the majority of cases of the iron overload syndrome Hereditary Hemochromatosis. (Bacon et al., 1999) Properly functioning HFE protein binds to the transferrin receptor and lowers its affinity for the iron transport protein transferrin. At least two HFE variant proteins (those from individuals with C282Y and H63D single nucleotide polymorphisms) have a reduced ability to perform this function. (Feder et al., 1998) Disruption of HFE function may increase transferrin mediated iron uptake in some tissues (Feder et al., 1998), and trigger a cascade of events that enhances iron absorption through intestinal transport proteins such as Divalent Metal Transporter-1 (DMT-1). (Pietrangelo, 2010; Byrnes et al., 2002; Brasse-Lagnel et al., 2011) Because cadmium can also be transported by these intestinal metal transporters (Gunshin et al., 1997; Vesey, 2010; Bressler et al., 2004), and can bind transferrin (Harris and Madsen, 1988), HFE variants may also alter cadmium absorption, toxicokinetics, or biomarker levels.

Previously, Akesson et al. reported that Hemochromatosis patients had increased blood cadmium levels, but only if they received regular phlebotomy treatments. (Akesson et al., 2000) The mechanism underlying these results is unclear but it may involve HFE and Transferrin function. Given the nearly ubiquitous nature of low level cadmium exposure (Jarup and Akesson, 2009; CDC, 2013) and the high prevalence of HFE (Merryweather-Clarke et al., 1997) and Transferrin (Lee et al., 1999) variants, research is needed to determine if these variants can alter cadmium absorption in the context of low iron or hemoglobin levels, even in the absence of clinical hemochromatosis. If carriers absorb more cadmium from food, they may be more susceptible to developing cadmium mediated health effects from chronic low level exposure. Thus carriers of these variants may represent sensitive subpopulations to consider in cadmium risk assessments.

In this study we evaluated whether common HFE and Transferrin variants: HFE C282Y, HFE H63D (Bradley et al., 1998), and Transferrin P570S (Lee et al., 1999) predicted cadmium levels among the participants in the Normative Aging Study (NAS). (Bell et al., 1972) We also considered interactions between these genotypes and several factors that have been previously shown to influence cadmium exposure, transport, or absorption, including: hemoglobin levels, intake of several nutrients, and smoking. (ATSDR, 2008; Fox et al., 1980; Andersen et al., 2004; Sarhan et al., 1986; Berglund et al., 1994)

2. Methods

2.1. Data source and study population

The Normative Aging Study (NAS) is a longitudinal cohort study started in 1963 at the Veterans Administration (VA) Outpatient Clinic in Boston MA. (Bell et al., 1972; Wang et al., 2007) Between 1963 and 1968, 2280 males between the ages of 21 to 80, were enrolled in the NAS. The study was intended to focus on the aging process in generally healthy adult males, so participants were screened prior to enrollment to assure that they were free of most chronic health conditions. These generally healthy participants were screened for conditions such as cancer, asthma, cardiovascular disease, gout, diabetes, hypertension, and peptic ulcers. (Jain et al., 2007) They were not screened for hemochromatosis, but we expect that not many (if any) would have been identified due to low penetrance of HFE mutations (Nadakkavukaran et al., 2012; Asberg et al., 2007), and the later life onset of symptomology in classic (HFE related) hemochromatosis. (Pietrangelo, 2010) Every 3 to 5 years NAS participants have medical and laboratory evaluations, and respond to health related questionnaires. Between June 1992 and May 2010, 756 participants (ages 51–97) provided toenail clippings which were analyzed for cadmium content. Over 96% of these measurements had toenail clipping dates listed and all of these dates were within one year of the NAS study visit. The new portion of the study (analyzed here), was approved by the Human Research Committees of the Harvard School of Public Health and the Department of Veterans Affairs Boston Healthcare System.

2.2. Genotyping

We genotyped participants for the following single nucleotide polymorphisms (SNPs): HFE C282Y (RS1800562), HFE H63D (RS1799945), and Transferrin P570S (RS1049296), using methods described previously. (Park et al., 2009) Briefly, we extracted DNA from white blood cells with PureGene Kits (Gentra Systems, Minneapolis MN, USA), and then amplified the SNPs along with 100 bp flanking sequences using multiplex polymerase chain reaction (PCR). Primers designed with SpectroDESIGNER software (Sequenom, San Diego, CA, USA), and primer sequences have been previously published (Park et al., 2009)

2.3. Cadmium biomarker

We assessed toenail cadmium levels as described previously. (Mordukhovich et al., 2012) Briefly, toenail clippings were collected from all toes and pre-cleaned by sonicating in 1% Triton X-100 followed by rinsing with distilled deionized water and drying at 60 °C for 24 h. Toenails were then weighed and dissolved in HNO_3 for 48 h at room temperature. The samples were diluted to up to 5 mL with deionized water and then analyzed with an inductively coupled plasma-mass spectrometer (DRC 11, Perkin Elmer, Norwalk CT). Indium was used as the internal standard. Quality control was ensured by analyzing a calibration verification standard [National Institute of Standard and Technology Standard References Material 1643e (trace elements in water, Gaithersburg, MD)], a 1 ng/mL mixed element standard solution, continuous calibration standards, and a procedural blank. Daily analytic variation was assessed with Certified Reference Material GBW 07601. Five replicate measurements were averaged to yield each toenail cadmium concentration. Toenail cadmium levels in this population likely reflect exposure from 10 to 18 months prior to the date of collection. This estimate is based on studies of occupational cadmium exposure (10–12 month lag), (Grashow et al., 2014) and the fact that toenail growth rates decrease with age. (Mordukhovich et al., 2012; Slotnick and Nriagu, 2006)

2.4. Statistical analysis

2.4.1. Genotype frequencies

We calculated allele and genotype frequencies among the 756 participants, and performed chi-square tests for specified proportions to compare the observed genotype frequencies with the expected genotype frequencies based on Hardy-Weinberg equilibrium. This serves as a quality control check, because deviation from Hardy-Weinberg equilibrium can be an indicator of genotyping errors or issues with data quality. (Hosking et al., 2004) An exact test was used for HFE C282Y because there were < 5 in the homozygous variant group.

2.4.2. Descriptive statistics

For our initial analyses we evaluated the first toenail cadmium measurement for each of the 756 participants (i.e. if a participant provided samples from multiple visit dates, we used only the first sample provided). We excluded 5 cadmium values due to insufficient weight of the toenail sample. Because the distribution of toenail cadmium concentrations was roughly lognormal, we log transformed cadmium values for *t*-test and regression analyses. Measurement imprecision resulted in some negative cadmium values. We did not left censor the data by eliminating values below the limit of detection, as this implicitly assumes that the sources of measurement error are distinct and incomparable on either side of this detection limit. (Whitcomb