Environment International 121 (2018) 365–374

Contents lists available at ScienceDirect

Environment International

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

Urinary excretion of phenols, parabens and benzophenones in young men: Associations to reproductive hormones and semen quality are modified by mutations in the Filaggrin gene

Ulla Nordström Joensen[a], Niels Jørgensen[a], Jacob P. Thyssen[b], Pal Bela Szecsi[c,d], Steen Stender[c], Jørgen Holm Petersen[a], Anna-Maria Andersson[a], Hanne Frederiksen[a,*]

[a] Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[b] National Allergy Research Centre, Department of Dermatology and Allergy, Copenhagen University Hospital Gentofte, Copenhagen, Denmark
[c] Department of Clinical Biochemistry, Copenhagen University Hospital Gentofte, Copenhagen, Denmark
[d] Department of Clinical Biochemistry, Copenhagen University Hospital Holbæk, Holbæk, Denmark

ARTICLE INFO

Handling editor: Lesa Aylward

Keywords:
Endocrine disrupting chemicals
Bisphenol A
Benzophenone-3
Testicular function
Filaggrin gene

ABSTRACT

Background: The filaggrin gene (*FLG*) encodes an epidermal protein, filaggrin, which is important for normal skin barrier functions. We previously showed that *FLG* loss-of-function mutation carriers have a higher internal exposure to some non-persistent chemicals such as certain phthalates and parabens, suggesting increased transepidermal penetration. Several groups of non-persistent chemicals are suspected endocrine disrupters with potential to affect testicular function.

Objectives: To investigate associations between exposure to non-persistent chemicals and testicular function in young Danish men with and without *FLG* mutations.

Methods: We measured urinary concentrations of bisphenol A (BPA) and other simple phenols, parabens, and UV filters including benzophenones (BP-1, BP-3 and 4-HBP) in men genotyped for *FLG* R501X, 2282del4, and R2447X loss-of-function mutations; in total 65 mutation carriers and 130 non-carriers (controls) were included. Outcomes were markers of testicular function, assessed by serum reproductive hormones and semen quality.

Results: We found that associations between urinary chemical concentrations and outcomes were different in cases and controls. Within the group of *FLG* mutation carriers, higher urinary concentrations of BPA, BP-1 and BP-3 were associated with higher testosterone and estradiol serum levels and lower FSH. Similar trends in hormone levels were observed for *FLG* mutation carriers with measurable levels of 4-HBP compared to those who had no detectable levels of urinary 4-HBP. Furthermore, those in the highest urinary BPA quartile had lower sperm motility than those in the lower quartiles. None of these associations were evident in the control group. In the control group, however, lower sperm motility and sperm concentration were observed in the men with detectable urinary 4-HBP compared to the men non-detectable urinary 4-HBP. We found no association between any parabens and outcomes, nor for the other measured phenols or UV filters.

Conclusions: Associations between male reproductive health parameters and urinary levels of BPA and benzophenones such as BP-3, BP-1 and 4-HBP were observed in *FLG* mutation carriers but not in controls from the same study population. This difference between *FLG* mutation carriers and non-carriers is not explained solely by differences in exposure levels of the examined compounds as e.g. BPA and 4-HBP urinary levels did not differ between the two groups. We hypothesise that effects of exposure to these compounds may be modulated in *FLG* mutation carriers by either different levels of co-exposures or by route of uptake, with a higher fraction of the uptake by dermal uptake.

1. Introduction

Potential endocrine disrupting chemicals such as bisphenol A (BPA) and triclosan (TCS), parabens and benzophenone-3 (BP-3) have been in focus during the past decades. We have previously shown that nearly all Danish children and adults are exposed to several of these chemicals (Frederiksen et al., 2014), some of which have been associated with altered testicular function in humans (Lassen et al., 2014). However,

* Corresponding author at: Department of Growth and Reproduction, Section 5064, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark.
E-mail address: hanne.frederiksen@regionh.dk (H. Frederiksen).

https://doi.org/10.1016/j.envint.2018.09.020
Received 17 May 2018; Received in revised form 11 September 2018; Accepted 11 September 2018

causality of these associations remains uncertain due to the observational nature of the human studies, and the results are inconsistent between studies (Den et al., 2015; Hauser et al., 2015; Lassen et al., 2014; Mendiola et al., 2010; Scinicariello and Buser, 2016).

Humans are continuously exposed to TCS, parabens and several UV filters/absorbers through cleaning products, personal care products, cosmetics and sun screens (Koch et al., 2014; Liao and Kannan, 2014; Xue et al., 2017). Parabens and UV filters are absorbed through skin by direct contact, or from air and clothing, and are detectable in both plasma and urine after topical application (Janjua et al., 2007; Janjua et al., 2008a; Janjua et al., 2008b; Morrison et al., 2017). Significant dermal uptake of BPA has also been suggested (Demierre et al., 2012; Ndaw et al., 2016; von Goetz et al., 2017).

Concentrations of these compounds measured in serum or urine varies widely between individuals. This variability most likely reflects differences in external exposure and an inter-individual variability in absorption, metabolism and clearance. Genetic factors, gender, lifestyle and other factors may all contribute to the variation. One candidate factor that could increase absorption of chemicals is dermal filaggrin deficiency. Filaggrin is a large protein that is crucial for normal skin barrier function by increasing hydration and lowering pH. Loss-of-function mutations in the filaggrin gene (*FLG*) (Smith et al., 2006) are present in approximately 9% of lightly-pigmented Europeans, and a slightly lower proportion of Asians (Irvine et al., 2011). These mutations can cause ichthyosis vulgaris (clinical condition hallmarked by dry scaly skin), and increase the risk of atopic dermatitis, food allergies, and allergic asthma (Irvine et al., 2011). Loss-of-function mutations in *FLG* facilitate transfer of allergens such as nickel and chromium, as well as other chemicals, across the epidermal barrier, and in particular in patients with atopic dermatitis (Halling-Overgaard et al., 2016; Thyssen et al., 2010b). Besides loss-of-function mutations in *FLG*, inflammation of the skin as occurs in manifest atopic dermatitis also reduces epidermal filaggrin (Thyssen et al., 2010a). Most individuals with loss-of-function mutation in *FLG* do not have clinical signs of skin inflammation, but their skin barrier function still is often reduced (Scharschmidt et al., 2009).

Filaggrin depleted skin, particularly in patients with atopic dermatitis, is often treated daily with moisturizing agents, often on large areas of skin. However, little is known about the absorption of additive chemicals from personal care products, or about the possible beneficial or harmful (side) effects of the additive chemicals. In young Danish men, we showed that carriers of *FLG* loss-of-function mutations had higher urinary concentrations of some phenols, parabens and UV filters (Joensen et al., 2017), and of several phthalates (Joensen et al., 2014). Furthermore, certain patterns of phthalate metabolites in urine were associated with male reproductive hormones (Joensen et al., 2012), but the increased phthalate exposure we found associated with *FLG* loss-of-function mutation carrier status, was not itself associated with semen quality or reproductive hormones in *FLG* mutation carriers (Joensen et al., 2014). This led us to hypothesise that the associations between chemical exposure and markers of male reproductive function may differ between *FLG* loss-of-function mutation carriers and non-carriers.

In the current study we explored the associations between urinary concentrations of phenols, parabens and benzophenone type of UV absorbers/filters with reproductive hormones and semen quality, and the influence of *FLG* mutation carrier status on these associations.

2. Materials and methods

2.1. Subjects and sample selection

The participants in the current study were Danish men from the general population, median age 19 years, unselected regarding reproductive function and possible skin disorders, who were attending a mandatory screening for military enrollment eligibility in the Copenhagen area. They were approached about an ongoing semen quality study, regardless of whether they were determined fit for military service. Details of that study have been published previously (Jørgensen et al., 2012). In short, the men who accepted participation provided a blood, urine, and a semen sample and answered a questionnaire, and underwent a physical examination, all on one day. The questionnaires focused on reproductive disorders and not skin or other atopic disorders; hence, no specific information was collected about diagnoses or use of medication for clinical skin conditions that could be related to filaggrin deficiency. Men participating in 2007–2009 were genotyped for *FLG* loss-of-function mutations, which were present in 65 of 861 participants, as reported in (Joensen et al., 2014). These 65 men constituted a "*FLG* loss-of-function" group, and for each of these 65 men, the two men without *FLG* loss-of-function mutations who had participated just before and after each case were retrospectively selected as controls, giving 130 controls. Thus, 195 men in total had urine samples analyzed for phenols, parabens and UV filters; the urinary concentrations have previously been published (Joensen et al., 2017). All analyses of urine, serum and semen were done within in the same study protocol for the whole group, and *FLG* status was unknown to both participants and lab technicians.

Fifty-one of these men (of whom 17 were *FLG* loss-of-function carriers) have been included in a previous publication on associations between testicular function and bisphenol A levels in 308 Danish men (Lassen et al., 2014).

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the regional ethical committee. All participants were informed about the study orally and in writing, and informed consent was signed individually prior to participation.

2.2. Chemical analyses

In total 24 chemicals (see Table 1 for full chemical names and abbreviations); BPA, TCS, TCC, 3 chlorinated phenols, 2 phenyl phenols, 7 parabens and 9 UV filters were analyzed in urine samples from the 195 men by isotope dilution on-line TurboFlow-liquid chromatography-tandem mass spectrometry (LC-MS/MS) with prior enzymatic deconjugation (Frederiksen et al., 2011; Frederiksen et al., 2013; Frederiksen et al., 2016). Samples were analyzed in four batches for each of the three analytical methods; except phenol analysis of the 51 samples collected from September 2008 to June 2009, which have been analyzed by same analytical methods for a previous study (Lassen et al., 2014). Each batch included standards for calibration curves, about 40–50 unknown samples, two blanks, two urine pool controls and two urine pool controls spiked with phenol, paraben or UV filter standards at low and high level. The inter-day variation, expressed as the relative standard deviation (RSD) was $\leq$10% for most analytes in both spike levels and the recovery of all analytes was > 85% except for TCS (77%). The levels of detection (LOD) are presented in Table 1.

2.3. FLG genotyping

DNA was purified from blood samples and stored at −80 °C until *FLG* genotyping, which was performed slightly modified according to a previously published method (Meldgaard et al., 2012). Briefly, three regions covering the mutations R501X, 2282del4, and R2447X of the *FLG* were asymmetrically amplified from genomic DNA by PCR using DNA tagged allele-specific primers. The obtained single-stranded PCR products were hybridized. The obtained PCR products were hybridized to spectral coded MAGPlex C microbeads (Luminex, Austin, Texas) carrying anti-tag DNA probes specific for the mutations and wild-type and subsequently analyzed on a BioPlex 200 (Bio-Rad, Hercules, California). The call rates were > 99% for all six alleles. Deviation from Hardy-Weinberg equilibrium was tested for all three *FLG* null variants using the free online calculator at the Online Encyclopedia for Genetic Epidemiology studies (http://www.oege.org/software/hwe-mr-