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Polymorphism in xenobiotic and estrogen metabolizing genes, exposure to perfluorinated compounds and subsequent breast cancer risk: A nested case-control study in the Danish National Birth Cohort

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ABSTRACT

In the present case-cohort study based on prospective data from Danish women, we aimed to estimate the main effect of polymorphisms in genes known to be involved in the steroid hormone metabolic pathway and xenobiotic metabolism on the risk of developing breast cancer. We also studied a possible effect measure modification between genotypes and levels of serum perfluoroalkylated substances (PFASs) on the risk to breast cancer. We have previously reported a weak association between serum PFASs levels and the risk of breast cancer for this study population of Danish pregnant nulliparous women as well as in a smaller case-control study of Greenlandic women.

The study population consisted of 178 breast cancer cases and 233 controls (tabnulliparous and frequency matched on age) nested within the Danish National Birth Cohort (DNBC), which was established in 1996-2002. Blood samples were drawn at the time of enrollment (6-14 week of gestation). Serum levels of 10 perfluorocarboxylated acids (PFCAs), 5 perfluorosulfonated acids (PFSAs) and 1 sulfonamide (perfluorooctane-sulfonamide, PFOSA) were measured. Genotyping was conducted for *CYP1A1* (Ile462Val; rs1048943), *CYP1B1* (Leu432Val; rs1056836), *COMT* (Val158Met; rs4680), *CYP17A1* (A1→ A2; rs743572); *CYP19A1* (C→ T; rs10046) by the TaqMan allelic discrimination method.

In overall, no significant associations were found between the investigated polymorphisms and the risk of breast cancer in this study among Danish women. The previously found association between PFOSA and risk of breast cancer did vary between different genotypes, with significantly increased risk confined to homozygous carriers of the following alleles: COMT (Met), CYP17 (A1) and CYP19 (C).

Conclusion:Our results indicate that polymorphisms in COMT, CYP17 and CYP19 which are involved in estrogen biosynthesis and metabolism can modulate the potential effects of PFOSA exposure on the development of breast cancer.

1. Introduction

Breast cancer is the most common cancer in women in Denmark, with an age-standardized incidence rate of 99.8 per 100,000 women year during year 2009-2013 (NORDCAN data base (Engholm et al. 2010)).

Many of the established risk factors for breast cancer are related to lifetime exposure to estrogens (Brown and Hankinson 2015). Apart from hereditary genetic factors e.g. mutations in the BRCA-1/2 genes, the risk is increased by early menarche, late menopause, obesity after menopause, alcohol intake, smoking and high fat intake (Fredslund and Bonefeld-Jorgensen 2012). Other factors that seem to reduce the risk are earlier age at first birth, the number of full term pregnancies, breastfeeding, and physical activity. However, the known risk factors only "explain" less than a third of all cases and the risk of breast cancer is probably modified by lifestyle and environmental exposures (Madigan et al. 1995; Rudolph et al. 2016).

Several candidate genes are of interest for their possible role in

Abbreviations: BMI, Body Mass Index; CI, Confidence intervals; COMT, Catechol-O-methyltransferase; CYP17, 17α-hydroxylase and 17, 20-lyase; CYP19, Aromatase; CYP1A1, Cytochrome P450 1A1; CYP1B1, Cytochrome P450 1B1; E2, Estradiol; Ile, Isoleucine; Leu, Leucine; Met, Methionine; RR, Relative risk; PFAS, perfluoroalkylated substance; PFHxS, perfluorohexane sulfonate; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate; PFOSA, Perfluorooctane sulfonamide; SNP, Single nucleotide polymorphism; Val, Valine

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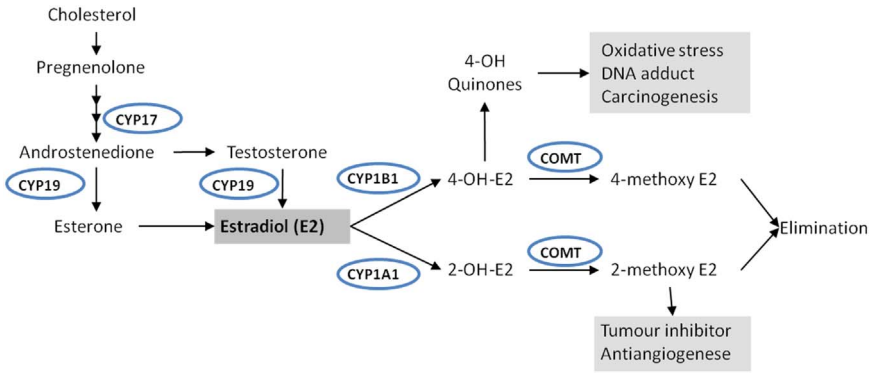

Fig. 1. Simplified schematic presentation of enzymes involved in estrogen biosynthesis and metabolism.

breast cancer risk, including members of cytochrome P450 (CYP). They are involved in synthesis and metabolism of estrogen as well as metabolism of xenobiotics. Variations including single nucleotide polymorphism (SNP) in these genes that might affect the circulating levels of estrogens and oxidized reactive intermediates are hypothesized to modulate the individual susceptibility to breast cancer risk. Such candidate genes include cytochrome P450 (CYP) 1A1, CYP1B1, Catechol-O-methyltransferase (COMT), 17α-hydroxylase and 17, 20-lyase (CYP17) and aromatase (CYP19) (Dumas and Diorio 2011; Ghisari et al. 2014) (Fig. 1).

CYP1A1 and CYP1B1 play important roles in the first step detoxification of xenobiotics as well as metabolism of estradiol to the 2-hydroxy and 4-hydroxy catechol estrogen metabolites (2-OH-E2 and 4-OH-E2), respectively. Metabolic activation of numerous pro-carcinogens such as polycyclic aromatic hydrocarbons by cyp1A1 generates reactive epoxide intermediates that might increase the risk of oxidative stress and cancer (Crofts et al. 1994; Nebert and Dalton 2006). At the same time, *CYP1A1* and *CYP1B1* genes are inducible by some environmental chemicals via the aryl hydrocarbon receptor (Safe 1994, 1995; Long and Bonefeld-Jorgensen 2012). The estrogen metabolite, 4-OH-E2, is highly estrogenic and carcinogenic in animal models (Newbold and Liehr 2000) and increased expression in neoplastic mammary tissue was observed (Liehr and Ricci 1996).

Several SNPs have been identified in *CYP1A1*, including the A to G transition at position 4889 in exon 7 resulting in a change from an isoleucine to valine amino acid (Ile →Val) at codon 462 (Cascorbi et al. 1996). This variant is reported to be significantly associated with CYP1A1 inducibility and higher enzyme activity (Cosma et al. 1993; Crofts et al. 1994) that might cause higher rates of carcinogen activation. In the CYP1B1 gene an important SNP (C→G) at codon 432 in exon 3 leads to an amino acid substitution of leucine to valine (Leu→Val), which increases the 4-hydroxylation activity of CYP1B1 by three fold (Shimada et al. 1999; Li et al. 2000). Despite the functional effects of some variants, the role of *CYP1A1* and *CYP1B1* in breast cancer development remains controversial. Several meta-analyses on women from different ethnicities with breast cancer report little to no evidence that genetic variation in *CYP1B1* influences breast cancer risk (Blackburn et al. 2015).

A major inactivation step for 2-OH-E2 and 4-OH-E2 catechol estrogens is the conversion to their non-genotoxic methoxy derivatives by the phase II enzyme COMT. In the *COMT* gene, a single G to A base pair change results in an amino acid change from valine to methionine (Val →Met) at codon 108 of the soluble form of COMT and codon 158 of the membrane-bound form of COMT. This amino acid change has been associated with 3- to 4-fold decrease in enzyme activity in vitro (Lotta et al. 1995; Dawling et al. 2001). Still, epidemiological data on the influence of the variant *COMT* (Met/Met) and the risk of human breast cancer are diverse (Ding et al. 2010; Mao et al. 2010).

CYP17 is involved in the early stages of estrogen biosynthesis by converting pregnenolone and progesterone to precursors of androgens and estrogens, respectively. A common single base pair substitution (−34T→C also called A1- > A2) in the 5′ untranslated region (5' -UTR) of *CYP17* creates an additional SP1-type (CCACC box) promoter site, which is suggested to up-regulate the expression of *CYP17* (Carey et al. 1994). However, molecular evidence did not support the suggested mechanism (Kristensen et al. 1999) and the functional impact of the T/C change is presently to our knowledge not known. Still, there are some reports indicating that the A2 allele correlates with higher serum levels of various sex steroids (e.g., testosterone, progesterone, estrone, and estradiol) mainly in premenopausal women (Carey et al. 1994; Feigelson et al. 1998; Haiman et al. 1999).

The final step of estrogen biosynthesis is catalyzed by CYP19, also called aromatase, which converts androgens (testosterone and androstenedione) to estrogenic steroids (estradiol and estrone) (Dumas and Diorio 2011). Aromatase has been found to be expressed at higher levels in breast tumors, and variations within the CYP19 gene may contribute to this higher expression (Singh et al. 2005)). A C→T base substitution in the 3´-UTR has been frequently studied, and the T allele has been associated with higher levels of postmenopausal circulating E2 (Dunning et al. 2004) and related to breast cancer risk in some ethnicities (Pineda et al. 2013).

Previously we conducted a nested case-control study within the Danish National Birth Cohort (DNBC) to examine the potential role of serum levels of perfluoroalkylated substances (PFASs) in premenopausal breast cancer. The perfluorinated alkyl acids are extremely persistent (Parsons et al. 2008) and resistant to metabolism in the human body, and have low elimination rate. The mean human serum half-lives has been estimated to be 2.3-3.5 years for PFOA, 5.4-6.7 years for PFOS and 7.7–8.5 years for PFHxS as studied in retired fluorochemical production workers followed over time (Olsen et al. 2007; Krafft and Riess 2015). In overall, we found a weak positive association between levels of perfluorooctane sulfonamide (PFOSA; adjusted RR 1.05, 95 % CI 1.00–1.09) and a weak negative association between levels of perfluorohexane sulfonate (PFHxS; adjusted RR 0.67, 95 % CI 0.45-1.01) in serum drawn early in their pregnancy and risk of breast cancer diagnosed 10-15 years later (Bonefeld-Jorgensen et al. 2014b). These associations were further strengthened upon stratification for age at diagnosis for women younger or 40 years of age and particularly in the highest 5th quintile of PFOSA (adjusted RR 3.42, 95 % CI 1.25–9.36).

PFOSA is a synthetic fluorocarbon compound with grease and water resistant properties used in food packaging and other consumer products, and breaks down to perfluorooctane sulfonate (PFOS) (Lehmler 2005). PFOS has been added to Annex B of the Stockholm Convention on persistent organic pollutants (Stockholm-Convention 2009). Several in vivo and in vitro studies have indicated that some PFASs may have the potential to disrupt endocrine homeostasis (Posner et al. 2013).

In the present study, these associations between serum levels of PFASs and breast cancer risk in the Danish women were further evaluated after stratification by genotypes for the assessment of