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PPARgamma-PGC-1alpha activity is determinant of alcohol related breast cancer

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ABSTRACT

Alcohol is a risk factor for postmenopausal breast cancer. One of several proposed mechanisms is that alcohol-related breast cancer is caused by increased sex hormone levels. PPARγ inhibits aromatase transcription in breast adipocytes. We reproduced previously found allele-specific effects of the wildtype Pro-allele of PPARG Pro[12]Ala in alcohol related breast cancer. In transiently transfected cells, transcriptional activation by PPARγ and the PPARγ-PGC-1α complex was inhibited by ethanol. PPARγ 12Ala-mediated transcription activation was not enhanced by PGC-1α, resulting in allele-specific transcription activation by the PPARγ 12Pro-PGC-1α complex.

Our results suggest that PPARγ and PGC-1α activity is an important determinant of alcohol related breast cancer.

1. Introduction

Alcohol is a well-known risk factor for breast cancer and alcohol intake is associated with a 7% increased risk of breast cancer pr.10 g/day [1,2]. It has been estimated that breast cancer constitutes 60% of the alcohol induced cancer worldwide [3]. Danish women have a high alcohol intake compared with women from other European countries [4] and breast cancer is by far the most common cancer form among women in the Western world including Denmark.

Ethanol and the primary metabolite acetaldehyde are both classified as human carcinogens by the International Agency for Research on Cancer (IARC). The mechanism behind alcohol related breast cancer is not clear [2]. Several possible mechanisms have been proposed: ethanol may increase the levels of sex hormones, or disturb the folate metabolism, whereas acetaldehyde may act as a mutagen [2]. Controlled experiments have demonstrated that alcohol intake leads to increased serum levels of estradiol up to 6 h after intake in premenopausal women [5]. Plasma levels of several hormones have been found to be positively associated with alcohol intake in population-based studies [6].

We have previously shown that homozygous carriers of the wild type Pro-allele of *PPARγ2* Pro[12]Ala, but not variant Ala-allele carriers, were at risk of alcohol related

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breast cancer [7]. Carriers of the ^{12}Ala-allele had lower risk of breast cancer and, in addition, did not have increased risk of alcohol related breast cancer (IRR:0.83, 95% CI: 0.67–1.04), whereas the risk of breast cancer among homozygous wildtype allele carriers increased with 20% pr. 10 g alcohol/day (CI: 1.08–1.35, $P < 0.005$) [7]. This observation suggested that PPARγ plays an important role in alcohol related breast cancer. The *PPARG* gene encodes the transcription factor peroxisome proliferator-activated receptor γ. PPARγ regulates adipocyte differentiation and the expression of many adipose-specific genes via binding of a heterodimer of PPARγ and RXR (Retinoid X receptor) to regulatory response elements in target gene promoters [8]. The *PPARG* Pro^{12}Ala substitution leads to a 30% reduction in target gene transcription by PPARγ2 [9]. The PPARγ2 isoform is primarily expressed in adipocytes [8]. PPARγ has been shown to inhibit transcription of aromatase, the rate limiting enzyme in estrogen biosynthesis, in primary breast adipocytes [10]. Genetic variation in *CYP19* encoding aromatase correlates with serum hormone levels [11], indicating that aromatase activity is rate limiting for sex hormone synthesis. The PPARγ-RXR heterodimer seems to inhibit aromatase transcription from promoter 1.3/II, which is used in breast adipose tissue fibroblasts, by forming a complex with PGC-1α [10,12]. This complex formation sequesters PGC-1α, preventing formation of the LRH-1-PGC-1α complex, which is proposed to activate aromatase transcription [12].

We hypothesized that the observed interaction between the polymorphism *PPARG* Pro^{12}Ala and alcohol intake in relation to breast cancer, i.e. the observation that homozygous carriers of the *PPARG* Pro12Ala Pro-allele have increased risk of breast cancer from drinking alcohol whereas variant allele carriers do not, is caused by an ethanol and PPARγ−PGC-1α-dependent increase in aromatase activity leading to increased estrogen levels. Furthermore, we hypothesized that two potentially functional mis-sense polymorphisms in *PPARGC1A* encoding PGC-1α [13,14] might interact with the studied *PPARG* polymorphism in relation to alcohol related breast cancer, and that the polymorphisms may modify the association between alcohol intake and serum hormone levels in postmenopausal women.

In this paper, we provide evidence that genetically determined differences in PPARγ and PGC-1α activity modify the association between alcohol consumption and breast cancer risk in a study group of 798 matched pairs of postmenopausal women with and without breast cancer nested in the Diet, Cancer and Health study. Furthermore, we show that transcriptional activation by PPARγ and by the PPARγ-PGC-1α complex is inhibited by ethanol, and that PPARγ 12Ala-mediated transcription activation is not enhanced by PGC-1α, resulting in allele-specific transcription activation.

Our results suggest the PPARγ and PGC-1α dependent association between alcohol and breast cancer in postmenopausal women may be explained by an allele-specific PPARγ-dependent effect of ethanol on aromatase expression.

2. Materials and methods

2.1. Subjects

The subjects for the present study were selected from the ongoing Danish "Diet, Cancer and Health" cohort study [15]. The present study group has been described in detail previously [16] and includes a previously described study group of 377 postmenopausal women with breast cancer and 377 matched controls [7,17–21]. In short, 79,729 women aged 50–64 years, born in Denmark, living in the Copenhagen or Aarhus areas and having no previous cancers at the time of invitation, were invited to participate in the study between December 1993 and May 1997. A total of 29,875 women accepted the invitation, corresponding to 37% of the invited women.

Cohort members were followed up for diagnosis of breast cancer from date of entry until either the date of diagnosis of cancer using record linkage to the Danish Cancer Registry until 2003 and afterwards by linkage to the Danish Pathology Databank, date of death, date of emigration, or April 27th, 2006, whichever came first. A total of 975 women were diagnosed with breast cancer during the follow-up period. For each case, one matched control was selected [16]. The control was cancer-free at the exact age at diagnosis of the case and was further matched on age at inclusion into the cohort (half-year intervals), use of hormone replacement therapy (HRT) (current/former/never) and on certainty of postmenopausal status (known/probably postmenopausal) upon inclusion into the cohort [16].

We excluded 90 pairs due to lack of blood samples or failed genotyping for either the case or the control, in addition 87 pairs due to missing information on confounders, leaving 798 pairs for study in this nested case-control study [16].

The Diet, Cancer and Health study and the present substudy were approved by the regional Ethical Committees on Human Studies in Copenhagen and Aarhus (jr.nr.(KF)11-037/01) and (jr.nr.(KF)01-045/93), and by the Danish Data Protection Agency.

2.2. Genotyping

Genotyping was performed on DNA isolated from frozen lymphocytes as described [22].Twenty ng of DNA was genotyped in 5 μl containing 1x Mastermix (Applied Biosystems), 100 nM probes, and 900 nM primers. Controls were included in each run, and repeated genotyping of a random 10% subset yielded 100% identical genotypes.

2.2.1. PPARG

Pro^{12}Ala (rs1801282) was determined as previously described [7]. *PPARG* Pro^{12}Ala for 377 of the 798 pairs have been published previously [7].

2.2.2. PPARGCIA

PPARGC1A Gly^{482}Ser, (rs8192678) was genotyped using the predesigned assay ABI assay C_1643192_20 (Applied Biosystems).