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PHARMACOKINETICS AND DISPOSITION

Caffeine N3-demethylation (CYP1A2) in a population with an increased exposure to polychlorinated biphenyls

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Abstract
Objective To investigate the CYP1A2 phenotype distribution in a population with an increased exposure to polychlorinated biphenyls (PCBs) that would likely induce an increased activity of this enzyme. Further, to investigate the effect of sex, smoking, and oral contraceptive use on the CYP1A2 activity.
Methods In 305 randomly selected Faroese residents aged 18–60 years, the CYP1A2 activity was determined following oral intake of a caffeine dose and subsequent determination of the urinary metabolites and calculation of the caffeine metabolic ratio (CMR). PCB exposure was assessed by measuring the serum concentration of major congeners.
Results The CYP1A2 phenotype distribution was unimodal. The CMR was significantly higher both in smoking men and in smoking women, independent of oral contraceptive use, as compared with non-smokers. Among non-smokers, the CMR was significantly higher in women not using oral contraceptives than in those using oral contraceptives; a similar difference could not be established among smokers. The CMR appeared higher in men than in women, but stratified analyses confirmed a significant sex-related difference only among smokers not using oral contraceptives. Overall, the mean CMR in Faroese was significantly higher compared with the mean CMR in Danish historical controls. No association was found with PCB exposure and individual PCB congeners, except for one of three dioxin-like congeners, in confounder-adjusted multiple regression analyses.
Conclusion The CYP1A2 phenotype in Faroese residents was unimodally distributed and showed the inducing effect of smoking and the inhibiting effect of use of oral contraceptives, but a sex-related difference was not apparent after confounder adjustment. There was no statistically significant association between CMR and PCB exposure.

Keywords CYP1A2 · Caffeine · N3-demethylation · Caffeine metabolic ratio · Polychlorinated biphenyls · Faroe Islands

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Introduction

CYP1A2 is one of the major members of the cytochrome P450 family and it accounts for about 13% of the total P450

content of the human liver [1, 2]. More than 20 clinically important drugs are partly or predominantly metabolized by this enzyme, including caffeine [2, 3]. CYP1A2 is also well known for its role in the metabolic activation of some environmental and food-borne carcinogens, including arylamines and heterocyclic amines, thereby producing reactive intermediates [4–6]. The enzyme activity is lower in subjects using oral contraceptives [7, 8], while cigarette smoking induces CYP1A2 [9–11]. In addition, CYP1A2 expression is highly inducible by certain dietary and environmental chemicals [6, 9, 12]. Accordingly, the wide inter-individual variation in CYP1A2 activity [6, 12–14] is likely due to external factors. Still, genetic polymorphisms can not be ruled out as a possible contributing factor. Some single nucleotide polymorphisms (SNPs) of the *CYP1A2* gene recently reported could perhaps have some functional significance [16]. While the **1C*, **1K*, **7* and **11* alleles have been shown to encode decreased activity enzymes, the **1F* allele encodes high inducibility of the enzyme [15–21].

Of major interest in regard to CYP1A2 induction, 2,3,7,8-tetrachlorodibenzeno-*p*-dioxin (TCDD) and related chemicals act through binding to the cytosolic arylhydrocarbon receptor (Ah receptor) [4, 22, 23]. These associations are mainly found in in vitro studies [4, 23] and only very few in vivo studies have examined the induction of CYP1A2 activity by exposure to TCDD and related persistent organohalogen pollutants (POPs) [24–27]. Among these chemicals, the polychlorinated biphenyls (PCBs) are common environmental pollutants that encompass congeners with and without TCDD-like activities [22, 28]. Due to the accumulation of PCBs in North Atlantic food chains, thereby causing high concentrations in pilot whales, the residents of the Faroe Islands, who consume whale blubber, are exposed to elevated levels of PCBs [29–32]. Serum-PCB concentrations in the residents of this Nordic fishing community range up to about 20 μg/g lipid [33], which is about 50-fold above the current US average [31, 34]. However, some Faroese do not eat whale blubber, and show low serum-PCB concentrations similar to those prevalent in the US [33]. In light of this wide range of exposures, the Faroe Islands constitute a unique setting highly appropriate for examining the issue of CYP1A2 activity in increased PCB exposure.

The main purpose of this study was therefore to investigate the distribution characteristics of the CYP1A2 phenotype in a population with an increased exposure to PCBs that would likely induce an increased activity of this enzyme. Stratified analysis with respect to the influence of sex, smoking, and oral contraceptive use on the CYP1A2 activity was performed. The CYP1A2 activity was assessed by the urinary caffeine metabolic ratio (CMR). The N3-demethylation of caffeine is catalysed solely by CYP1A2, and we therefore calculated the activity-dependent ratio between caffeine metabolites excreted in urine following the ingestion of a single dose of caffeine [35].

Materials and methods

Subjects

From the Faroese National Register of residents we received the names of 900 randomly selected individuals between 18 and 60 years. Of these, approximately one third were excluded based on criteria that included daily medication, pregnancy and breast-feeding, known allergy to medications, and acute or chronic illness requiring treatment. One third refused to participate for various reasons. The 312 healthy volunteers recruited for the study comprised 144 females and 168 males. At the time of the examination, four participants admitted that they were currently taking medication (other than oral contraceptives), but these four subjects were not excluded, since the medications were known not to induce or inhibit the CYP1A2 activity.

Out of the 312 volunteers, five were excluded from CYP1A2 activity analysis: two women because of uncertain pregnancy status, and three individuals due to missing blood sample. Of the remaining 307 volunteers (139 women and 168 men), 128 were smokers. Of the women, 36 used oral contraceptives and four used other hormone treatment. For the PCB analysis, two persons were excluded due to lack of blood sample or insufficient serum, and serum-PCB was therefore available from 143 women and 167 men. Data for CYP1A2 activity *and* PCB exposure were available from 305 subjects (139 women and 166 men).

The Ethical Review Committee covering the Faroe Islands approved the protocol as a part of a project on "Drug metabolism among Faroese: the interaction between pharmacogenetic and environmental factors". Each volunteer provided informed written and verbal consent.

Experimental protocol

The subjects were instructed to abstain from ingesting methylxanthine-containing foods and beverages and medication from 24 hours before the caffeine dosage until the end of the urine collection 12 hours later. After voiding the bladder, each subject ingested 200 mg of caffeine (supplied by the Central Pharmacy, Odense University Hospital, Denmark). Approximately 4–6 hours later, each subject provided a urine sample in a conical tube containing 1 mL 1 M HCl. The urine sample was stored at −80°C until analysis. At the same occasion, measurements were conducted in regard to phenotypes for sparteine (CYP2D6) and mephenytoin (CYP2C19) oxidation polymorphisms by combined administration of 100 mg sparteine and 100 mg

mephenytoin [36]. Simultaneous administration of low doses of these drugs does not influence the outcome of their respective metabolic indices [37]. A questionnaire was used to obtain information about smoking status, use of oral contraceptives and monthly consumption of pilot whale and blubber.

Analysis of urine metabolites–caffeine metabolic ratio

The urine was analyzed by a high-pressure liquid chromatography method previously described [38] to obtain concentrations of the caffeine metabolites 5-acetylamino-6-formylamino-3-methyluracil (AFMU), 1-methyluric acid (1MU), 1-methylxanthine (1MX) and 1,7-dimethyluric acid (17DMU). The CYP1A2 activity was estimated by calculating the urinary caffeine metabolic ratio (CMR) from the N3-demethylation of caffeine in urine, i.e. (AFMU+1MX+1MU)/17DMU [35].

Analysis of serum-PCB

A 10-ml blood sample was drawn in a Venoject tube without additives (Terumo Europe, Rome, Italy). After a minimum of half an hour, the tube was centrifuged at 4000 g (Hettich Zentrifugen Rotina 35R, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) to provide about 5 ml serum. The serum was kept frozen at −80°C until analysis.

The major PCB congeners, PCB-101, PCB-105, PCB-118, PCB-138, PCB-153, PCB-156, and PCB-180 were determined on an Agilent 6890 Plus Gas Chromatographic system with electron-capture detection [39]. Separation was performed on a dual column system with a HP5-MS as the primary column and a DB-XLB column (30 m, 0.25 mm ID, 0.25 μm film thickness) as a secondary and confirmatory column (Agilent Technologies, Palo Alto, CA, USA). Extraction of the compounds from serum was performed with Solid-Phase Extraction. In brief; the SPE cartridge (Accubond II ODS-C18 columns, 200 mg, Agilent Technologies, Wilmington, DE, USA) was conditioned with isooctane, methanol and water. An aliquot of 600 μl serum was diluted into 600 μl Milli-Q treated water. Proteins were digested by adding 300 μl concentrated formic acid, followed by whirl mixing and ultrasonic treatment for 5 min. An aliquot of 1250 μl of the mixture (corresponding to 500 μl serum) was transferred to a preconditioned SPE cartridge and eluted slowly through the column by use of vacuum. Washing procedure with 2.5 ml water was followed by elution with 2×2.5 ml isooctane. The eluted phase was transferred to a preconditioned Florisil cartridge (Accu-BOND, 200 mg, Agilent Technologies, Wilmington, DE, USA), and eluted further with 2.5 ml of a mixture of 15% *tert*-butylmethylether and 85% petroleum benzine. The eluent was collected in a tarred centrifugation tube and evaporated at 30°C under a gentle flow of nitrogen until approximately 100 μl remained. The tube was weighed and the residue adjusted to a volume of 250 μl with isooctane. An aliquot of 4 μl was injected into the GC.

The PCB results were adjusted for total serum lipid content and reported as μg per gram lipid. The median limit of detection was 0.03 ppb for all congeners, which, at a mean lipid concentration of 10.3 g/l, corresponds to 0.0029 μg/g lipid.

Data analysis

The skewed distribution of the CMR was normalized by logarithmic transformation. Subgroups stratified according to major predictors (sex, smoking habits, and the use of oral contraceptives) were compared by *t*-test for equality of means. Further, the mean CMR was compared by a *t*-test with the mean CMR obtained in Danish historical controls [13].

Because PCB is a mixture of several congeners, we calculated the ∑PCB as the sum of PCB138, PCB153 and PCB180 multiplied by 2, because the sum of these three major congeners represents about 50% of the total PCB concentration in Faroese milk and serum [30, 33, 40]. Second, the estimated dioxin-like activity of the PCBs, indicated by TCDD equivalents (TEQs), was computed for the three mono-*ortho* substituted congeners PCB-105, PCB-118 and PCB-156 according to international guidelines [41]. In addition, results for individual congeners were also included in the calculations. Because of skewed distributions, the PCB results were log-transformed before statistical analysis. Non-detectable levels of PCB congeners were assumed to equal 0.001 μg/g lipid for the purpose of log transformation and statistical analysis; this level corresponds to about one third of the detection limit.

We performed multiple linear regression analyses to examine the association of the log-transformed CMR with log-transformed serum-PCB adjusted for the effect of potentially confounding variables (sex, age, smoking and oral contraceptive use). Further, stratified regression analyses were also carried out for relevant subgroups. In all analyses, we allowed for an interaction between sex and smoking due to the stronger effect of smoking status on enzyme activity in men than in women.

All two-sided p-values <0.05 were considered to be significant. The statistical analysis was carried out by use of the SPSS program package, version 13.0.

Results

The CMR showed a unimodal distribution with a geometric mean of 6.5 without any sign of outliers (Fig. 1). The values ranged from 1.1 to 59.5.

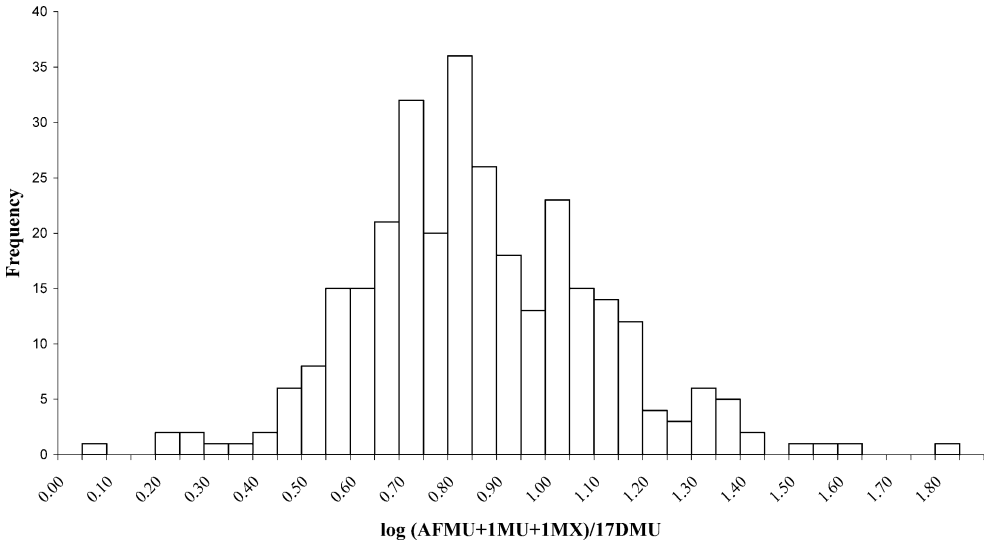

Fig. 1 Log-transformed distribution of the urinary caffeine metabolic ratio (CMR) reflecting CYP1A2 activity in Faroese men (n=168) and women (n=139)

The CMR was higher in men than in women (p<0.001). However, as shown in Table 1, if all smokers and oral contraceptive users were excluded, this difference disappeared (p=0.56). A significant sex-related difference was seen in regard to smoking after exclusion of oral contraceptive users (p<0.001). As compared to non-smokers, the CMR was increased in smoking men (p<0.001) and, to a lesser degree, in smoking women not using oral contraceptives (p=0.032). Further, a statistically significant increase in CMR was seen among smoking women using oral contraceptives compared with non-smoking women using oral contraceptives (p=0.001). In women not using oral contraceptives the CMR was significantly higher than in women using oral contraceptives (p=0.001), although significant only for non-smokers (p=0.95 for smokers).

The mean CMR of this study (mean±SD, 7.8±5.8; n=307) was compared by a t-test with the mean CMR of Danish historical controls (mean±SD, 5.9±3.4; n=378) [13]; a strongly significant difference (p<0.0001) was found.

The results of the serum analyses of seven PCB congeners, along with the ∑PCB and the mono-*ortho* TEQ are shown in Table 2. Results below the detection limit constituted a substantial problem for PCB-101 (73 samples), and PCB-105 (29 samples).

Smoking and use of oral contraceptives were significant predictors of the CMR in the multiple regression analysis (Table 3). Even though sex was not a significant confounder, it was included in the final model to achieve the best possible adjustment and because sex in other studies was

Table 1 Effect of gender, smoking and oral contraceptive use on CYP1A2 activity, expressed as geometric mean, interquartile range (25%–75%) and range of the caffeine metabolic ratio (CMR) in Faroese men (n=168) and women (n=139)

	Men			Women				
	All	Non-smokers	Smokers	All	− oral contraceptives		+ oral contraceptives	
					Non-smokers	Smokers	Non-smokers	Smokers
Number	168	100	68	139	56	43	23	17
Median age	38	39	35.5	39	40.5	44	36	33
Geometric Mean	7.3[d]	6.1[b]	9.4b[c,e]	5.7[a]	5.8[e,h]	7.3[b,f]	3.5[f,g]	5.5[h]
Interquartile range (25%–75%)	5.1–10.1	4.5–8.0	6.6–13.1	4.0–8.2	3.9–8.1	5.0–10.2	3.2–4.3	4.3–6.7
Range	1.8–59.5	1.8–33.9	2.7–59.5	1.1–29.8	1.6–20.6	1.5–29.8	1.1–6.8	3.3–13.7

Significantly different from (p<0.05):
[a] men
[b] men, smoking
[c] men, non-smoking
[d] women
[e] women, smoking, not oral contraceptives users
[f] women, non-smoking women, not oral contraceptives users
[g] women, smoking, oral contraceptives users
[h] women, non-smoking, oral contraceptives users

Table 2 Serum concentration (μg/g lipid) of PCBs in Faroese men (n=167) and women (n=143)

Congener	N	Geometric mean	Interquartile range (25%–75%)	Total range
PCB-101	310(44[a]/29[b])	0.007	0.003–0.02	0.001–0.1
PCB-105	310 (24[a]/5[b])	0.017	0.01–0.04	0.001–0.5
PCB-118	310	0.097	0.05–0.2	0.004–1.9
PCB-138	310	0.381	0.2–0.4	0.03–5.3
PCB-153	310	0.550	0.3–1.1	0.04–8.7
PCB-156	310 (1[b])	0.046	0.02–0.1	0.002–0.6
PCB-180	310	0.342	0.2–0.7	0.02–5.0
Total PCBs	310	1.468	0.7–2.9	0.1–21.8
∑PCB[c]	310	2.578	1.3–5.0	0.17–37.4
TEQ[d]	310	3.515	1.6–7.1	0.18–54.5

[a] number of samples with non-detectable level
[b] number of samples with level below the detection limit
[c] ∑PCB is calculated as 2.0×PCB (138+153+180)
[d] TEQ is calculated as ((PCB105+PCB118+5∗PCB156)∗10). Unit: pg TEQ/g lipid

found to be a significant confounder. Age was not associated with the CMR and was therefore left out of the final model.

PCB was weakly associated with the CMR (Fig. 2). Table 4 shows the results of the multiple regression analysis of changes in the log-transformed CMR values as an effect of log-transformed serum concentrations of the seven individual PCB congeners, ∑PCB and TEQ. After adjusting for the confounders, significant association with CMR was observed only for PCB-105, while PCB-118 showed an association of borderline significance. The regression equation explained only about 20% of the total variability of the CMR (R^2=0.20).

The Faroese men had significant higher ∑PCB concentrations (geometric mean, 3.44 μg/g lipid) than the Faroese women (geometric mean: 1.84 μg/g lipid) (p<0.001) and this difference was apparent among all PCB congeners analyzed (data not shown). Smoking was not associated with the ∑PCB concentrations in serum (p=0.35). Women who used oral contraceptives had a lower ∑PCB concentration (p=0.035) than the women who did not use oral contraceptives, but after adjusting for age this difference disappeared. However, stratified regression analysis in the subgroups (Table 1) did not reveal any interaction between confounders and serum-PCB concentrations in regard to the CMR values (data not shown).

Table 3 Multiple regression analysis of log-transformed CMR values on background variables in Faroese men (n=166) and women (n=139). R^2=0.20

Background variable	Effect[a]	p-value
Gender (women/men)	−7%	0.351
Smoker (yes/no) - men	56%	0.001*
Smoker (yes/no) - women	35%	0.001*
Oral contraceptives[b] (yes/no)	−34%	0.000*

[a] Percentage change in CMR for a specific change in each of the covariates
[b] Women only (n=139, 40 users)
* Statistical significant (p<0.05)

Discussion

This study is the first to examine the distribution characteristics of the CYP1A2 phenotype in the Faroe Islands, and it extends previous studies of CYP2D6 and CYP2C19 poor and extensive metabolizers and the distribution of *CYP2C8* and *CYP2C9* genotypes in the Faroese population [36].

The unimodal distribution is in accordance with some studies [13, 38], whereas in others a bimodal [10, 14] or even a trimodal distribution appears [12]. The distribution of CMRs in the Faroes would argue against the occurrence of genetic heterogeneities alone being responsible for the higher CYP1A2 activity. The variability is therefore likely to be due to external factors.

The associations of the CYP1A2 activity with suspected predictors are in accordance with expectations, with the exception of the absence of a sex-related effect. We confirmed previous findings that the CMR is increased in smokers [9–11]. Likewise, the CYP1A2 activity was lower in women using oral contraceptives than in women not using oral contraceptives, an effect thought to be due to an inhibition of CYP1A2 by the hormones [7, 8]. The disappearance of a significant sex-related difference in CMR between non-smoking subjects, when oral contraceptive users were excluded, is in agreement with findings in some studies [10, 35], whereas sex-related difference in stratified analyses has been found in other studies [9, 13].

The CMR among Faroese was higher than expected. As the CMR reflects the CYP1A2 activity, the higher CMR among Faroese indicates that CYP1A2 activity among Faroese in general is induced. The percentage of smokers in the present study was approximately 42%, as compared to only 21% in the Danish historical controls [13]. Although smoking could be suspected to be a cause of higher CMRs in the Faroese, an increased CMR was observed also in most non-smoking subgroups (Table 1), thus indicating that smoking cannot explain the higher CMR found among the Faroese. None of the other known predictors seem to be of importance in this regard.

Despite the relatively high serum-PCB concentrations in this population, no significant association with CMR was found, except for the dioxin-like congener PCB-105. These results suggest that the high CYP1A2 activity is not due to

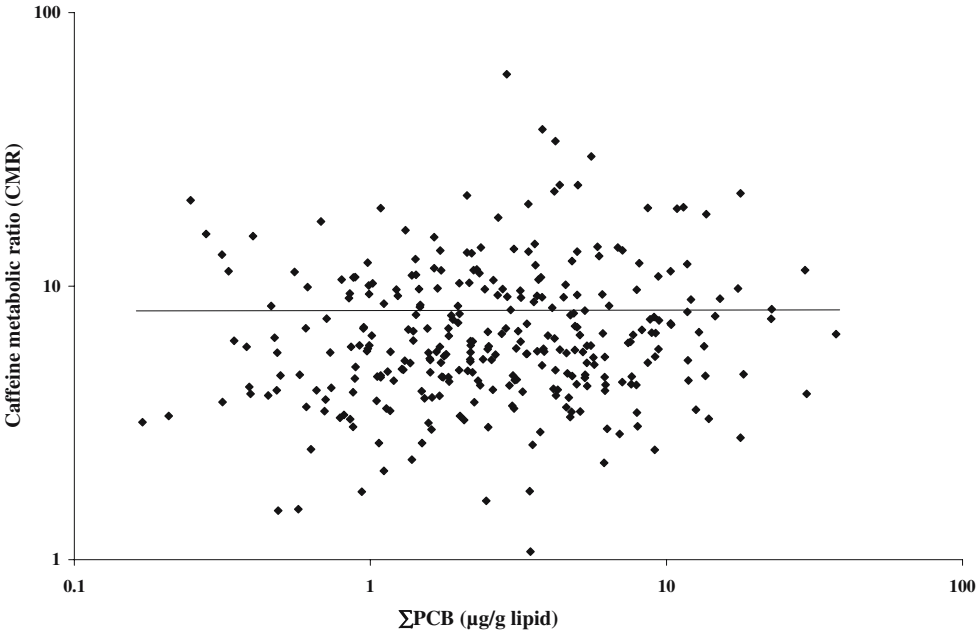

Fig. 2 Association between caffeine metabolic ratios (CMR) and serum ∑PCB concentrations in Faroese men (n=166) and women (n=139), using a logarithmic scale for both variables. r^2 for the linear regression line is 0.013

major PCB congeners. However, we did not determine coplanar congeners and dioxins, which are more potent inducers of the CYP1A2 enzyme. Analyses of dioxin-related compounds in the Faroes have shown low concentrations similar to those found elsewhere in northern Europe [40], and they, too, are therefore not likely explanations for an increased CYP1A2 activity. However, the mono-*ortho* congeners would be expected to induce both CYP1A and CYP2A family [22], and support is found in the correlation between PCB-105 and PCB-118 and the CMR, although no such correlation was found for PCB-156 and the TEQ-based PCB concentration, despite a high intercorrelation between the congeners. However, these observations may have been affected by low or non-detectable concentrations in some of the subjects examined.

Previous studies have reported equivocal findings in regard to associations between POPs and CYP1A2 activity, although few studies have investigated this association in vivo. One study found substantially induced hepatic CYP1A2 activity after high, but not moderate, TCDD exposure [26]. No significant association was observed between CMR and serum-TCDD levels among 58 workers previously exposed to TCDD and with relatively high current TCDD concentrations [25]. In subjects with environmental PCB exposure, a weak positive association between the serum concentration of ∑PCB and caffeine breath test (CBT) was observed [24]. Further, a significant correlation was also found with the PCB-based TEQ and even with individual di-*ortho* congeners not known to induce this enzyme [24]. The exposure levels in this study were in general lower than among the Faroese.

The validity of methods to assess the CYP1A2 activity also needs to be considered [27, 42]. Although the most appropriate caffeine metabolite in urine has been a matter of discussion [12, 42, 43], the CMR as applied in the present study correlates well with caffeine clearance rates [35] and appears to be a reliable measure of CYP1A2 activity [38]. The results obtained by CMR are similar to the CBT [42], but one study showed only correlation with hexabromobiphenyl exposure when using CBT, not CMR, as a measure of the CYP1A2 activity, despite a good correlation (r^2= 0.54, p<0.001) between these two methods [27]. Although the CMR may represent a reduced or dampened picture of the true magnitude of CYP1A2 variation, the rank order is not affected [42]. Further, the CMR is a very practical test and clearly sufficiently sensitive to detect the effects of smoking and oral contraceptive use. The CMR should therefore also be sensitive enough to reflect any substantial effect of PCB exposure, given the wide range of exposure levels present in the Faroese subjects examined.

Table 4 Confounder-adjusted multiple regression analysis of log-transformed CMR values on log-transformed serum-PCB concentrations in Faroese men (n=166) and women (n=139)

Congener	Effect[a]	p-value
PCB-101	−8%	0.194
PCB105	−12%	0.015*
PCB-118	−11%	0.068
PCB-138	1%	0.928
PCB-153	0%	0.966
PCB-156	1%	0.841
PCB-180	4%	0.570
∑PCB	1%	0.843
TEQ	−4%	0.603

[a] Percentage effect on CMR for every 10-fold increase in PCB

The regression equation explained only a small part of the total variability of the CMR, and the CYP1A2 activity is therefore affected by factors other than smoking and use of oral contraceptives. A previous twin study [13] suggested that the CMR was mainly under genetic control, and that the heritability was 0.725. Isolated populations, such as the Faroese, may have deviant gene frequencies, and specific genetic factors could therefore contribute to the higher average ratio and greater variability, but the unimodal distribution argues against major polymorphisms of the *CYP1A2* gene. However, polymorphisms that control induction of the gene would be possible, since each induction factor might cause only a slight shift in enzyme activity. If PCB-related induction is under genetic control, then the association between PCB exposure and the CMR would be less clear in the presence of heterogeneities.

The increased average CMR in the Faroese population is likely to be of clinical significance. Individual variation in activity is known to influence the effectiveness of prescribed medications and the risk of drug interactions [44]. An increased CYP1A2 activity could result in more frequent drug treatment failures among patients taking medications that are CYP1A2 substrates. Thus, of 27 drugs that were particular frequent causes of adverse drug reactions, 12 were metabolized by the CYP1A2 enzyme [44]. Although only a minor enzyme for several drugs, except for imipramine and theophylline, these results emphasize the important role of this enzyme in adverse drug reactions [44]. In addition, the increased enzyme activity may affect detoxification rates for toxic substances and could cause a higher cancer risk [12, 45, 46].

The significantly lower serum-PCB concentrations among the Faroese women compared to men may be interpreted as an effect of the "Diet recommendation concerning pilot whale meat and blubber" issued by the Food and Environmental Agency in the Faroes in 1998, that girls and women should not eat blubber until they have given birth to their children.

In conclusion, the expected effects of smoking and oral contraceptive use on CMR were confirmed, but an independent sex-related difference was not apparent. Compared with Danish historical controls, the mean CMR in the Faroes was significantly increased. No statistically significant association between CMR activity and PCB exposure was found. Because an increased enzyme activity has clinical consequences, these findings deserve attention. Further studies are warranted on possible polymorphisms of the *CYP1A2* gene among the Faroese.

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