CYP3A7*1C allele is associated with reduced levels of 2-hydroxylation pathway oestrogen metabolites

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Background: Endogenous sex hormones are well-established risk factors for breast cancer; the contribution of specific oestrogen metabolites (EMs) and/or ratios of specific EMs is less clear. We have previously identified a CYP3A7*1C allele that is associated with lower urinary oestrone (E1) levels in premenopausal women. The purpose of this analysis was to determine whether this allele was associated with specific pathway EMs.

Methods: We measured successfully 12 EMs in mid-follicular phase urine samples from 30 CYP3A7*1C carriers and 30 non-carriers using HPLC-MS/MS.

Results: In addition to having lower urinary E1 levels, CYP3A7*1C carriers had significantly lower levels of four of the 2-hydroxylation pathway EMs that we measured (2-hydroxyestrone, \(P = 1.1 \times 10^{-12}\); 2-hydroxyestradiol, \(P = 2.7 \times 10^{-7}\); 2-methoxyestrone, \(P = 1.9 \times 10^{-12}\); and 2-methoxyestradiol, \(P = 0.0009\)). By contrast, 16α-hydroxylation pathway EMs were slightly higher in carriers and significantly so for 17-epiestriol (\(P = 0.002\)).

Conclusions: The CYP3A7*1C allele is associated with a lower urinary E1 levels, a more pronounced reduction in 2-hydroxylation pathway EMs and a lower ratio of 2-hydroxylation:16α-hydroxylation EMs in premenopausal women. To further characterise the association between parent oestrogens, EMs and subsequent risk of breast cancer, characterisation of additional genetic variants that influence oestrogen metabolism and large prospective studies of a broad spectrum of EMs will be required.

Endogenous sex hormones are well-established risk factors for breast cancer. Pooled analyses of data from prospective studies have estimated that a doubling of circulating estradiol (E2), free E2 or E1 is associated with a 20–30% or 30–50% increase in breast cancer risk in pre- and postmenopausal women, respectively (Key et al., 2002, 2013). The contribution of specific oestrogen metabolites (EMs) to breast cancer risk is less clear. Briefly, interconversion between the parent oestrogens, E2 and E1 occurs by reversible oxidation at the 17α-position of the steroid ring; conversion of parent oestrogens to EMs is by irreversible hydroxylation at the 2-, 4- or 16-positions (Figure 1; Badawi et al, 2001; Tsuchiya et al, 2005; Samavat and Kurzer, 2015). A recent review, summarising evidence from four prospective studies of oestrogen metabolism and breast cancer risk concluded that there was consistent evidence that enhanced 2-hydroxylation was associated with a reduction in risk of breast cancer that was independent of the strong positive associations of unconjugated parent oestrogens (E2 and E1) with breast cancer risk (Ziegler et al, 2015).

In an analysis of single-nucleotide polymorphisms (SNPs) tagging genes that are involved in oestrogen synthesis and

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metabolism, we identified one SNP, rs10273424, which was associated with a 22% reduction in levels of urinary E1 glucuronide (E1G) in premenopausal women (Johnson et al, 2012). rs10273424 maps to the cytochrome P450 3A (CYP3A) gene cluster at 7q22.1; the CYP3A genes (CYP3A5, CYP3A7 and CYP3A4) encode enzymes that catalyse the oxidative metabolism of a wide range of exogenous and endogenous substrates including parent oestrogens (E1 and E2; Figure 1). The metabolic capacity of the CYP3A enzymes differ, depending on the substrate (Williams et al, 2002); with respect to oestrogen metabolism specifically, 2-hydroxylation of E1 to 2-OHE1 is catalysed by CYP3A4, 4-hydroxylation to 4-OHE1 is catalysed by CYP3A4 and CYP3A5 and 16α-hydroxylation to 16α-OHE1 is catalysed by CYP3A4, CYP3A5 and CYP3A7 (Figure 1; Badawi et al, 2001; Lee et al, 2003; Tsuchiya et al, 2005). Fine-mapping of the 7q22.1 association signal for urinary E1G levels implicated the CYP3A7*1C allele as the causal allele (Johnson et al, 2016). This allele, which comprises seven highly correlated single base changes mapping to the CYP3A7 promoter, results in expression of the fetal CYP3A7 gene in adult carriers of the CYP3A7*1C allele (Gonzalez, 1988; Kuehl et al, 2001; Burk et al, 2002).

The purpose of this current analysis was to determine whether, in addition to the association between CYP3A7*1C carrier status and lower urinary E1G levels, the CYP3A7*1C allele was associated with a reduction in levels of specific pathway EMs.

**MATERIALS AND METHODS**

**Study population.** The study population from which the women we included in this analysis were drawn has been described previously (Johnson et al, 2012). Briefly, they comprised 729 premenopausal women who were first-degree relatives and friends of breast cancer cases participating in the British Breast Cancer study (Johnson et al, 2005) or participants in the intervention arm of a trial of annual mammographic screening in young women conducted in Britain (Mammography Oestrogens and Growth Factors Study; Walker et al, 2009). To be eligible, women had to be having regular menstrual cycles, not using hormone replacement therapy or oral contraceptives and not to have been diagnosed with breast cancer at recruitment to the study. All women were of

Figure 1. Steroid hormone synthesis and endogenous oestrogen metabolism in humans. The 14 oestrogen metabolites (EMs) formed by hydroxylation of parent oestrogens (E1 and E2), at the 2-, 4- and 16α-positions of the carbon ring that were measured are shown within the red box. Enzymes (cytochrome P450 (CYPs) and catechol O-methyltransferase (COMT)) involved in oestrogen metabolism are in red. E1G, measured in our previous analysis (Johnson et al, 2012), is an E1 conjugate present in urine. The E1 measured in this analysis (after hydrolysis of glucuronide and sulphate conjugates in the first step of the LC-MS/MS protocol) is highly correlated with E1G (Spearman’s correlation, ρ=0.70, P<0.0001). Abbreviations: E1 = oestrone; E2 = oestradiol; 2-OHE1 = 2-hydroxyoestrone; 2-OHE2 = 2-hydroxyoestradiol; 2-MeOE1 = 2-methoxyoestrone; 2-MeOE2 = 2-methoxyoestradiol; 3-MeoE1 = 3-methoxyoestrone; 4-OHE1 = 4-hydroxyoestrone; 4-MeOE1 = 4-methoxyoestrone; 4-MeOE2 = 4-methoxyoestradiol; 16α-OHE1 = 16α-hydroxyoestrone; E3 = estriol; 16-KetoE2 = 16-ketoestradiol; 17-epiE3 = 17-epiestriol and 17β-HSD = 17β-hydroxysteroid.
self-reported White British ancestry. To be included in the original analysis and this subsequent analysis, women had to have provided serial urine samples (six follicular phase and one luteal phase), at pre-specified days of their menstrual cycle for measurement of creatinine adjusted urinary E1G using an in-house enzyme-linked immunosorbent assay (Johnson et al, 2012). To maximise the statistical efficiency of this analysis of the CYP3A7*1C allele, which has a minor allele frequency (MAF) of just 4% in Northern European populations (Johnson et al, 2016), we selected 60 women on the basis of genotype; a random sample of 30 CYP3A7*1C carriers and 30 CYP3A7*1C non-carriers. We further selected the two periovulatory samples (samples three and four of the six sequential follicular phase samples) on the basis that oestrogen levels would be at their peak in these samples and this would maximise any differences in levels between CYP3A7*1C carriers and non-carriers. To minimise random variation, we used the average of these two sequential samples as our outcome variable.

Ethics. The study was conducted in accordance with the tenets of the Declaration of Helsinki and all participants provided written informed consent.

Genotyping. Genotyping of the tag SNP rs45467892, which is perfectly correlated with the CYP3A7*1C allele, has been described previously (Johnson et al, 2016). Briefly, genotyping was by Taqman (Life Technologies, Paisley, UK). The call rate was 96.9% and concordance between duplicate samples was 100%.

HPLC-MS/MS analysis. LC-MS/MS analysis was carried out for 14 EMs using the method of (Xu et al, 2005, 2007). We were unable to measure one of the 15 EMs measured by (Xu et al, 2005, 2007) (16-Epiestriol), as there was no commercially available standard for this EM.

Briefly, two aliquots of frozen urine per woman were sent to the Mass Spectroscopy Facility for Quantitative Analysis, Faculty of Natural Science, Imperial College, London, for analysis. Hydrolysis of glucuronide and sulphate-conjugated EMs to form free EMs was carried out by mixing 500 μl of freshly thawed urine with 20 μl of internal standards (comprising 2 ng of each of five deuterium-labelled oestrogen metabolites (d-EMs); 17β-estradiol-d4, estriol-d3, 2-hydroxy-17β-estradiol-d5, 2-methoxy-17βestradiol-d5 and 16-epi-estradiol-d3; Qmx Laboratories Ltd, Dunmow, UK) and 500 μl of freshly prepared enzymatic hydrolysis buffer (100 μl of β-glucuronidase from Helix pomatia (Type H-2; Sigma-Aldrich, St Louis, MO, USA) in 10 ml 0.15 M sodium acetate buffer, pH 4.6 containing 2 mg of ascorbic acid). Samples were incubated at 37 °C overnight before extraction with dichloromethane and dansyl chloride derivatisation. The final derivatised samples (200 μl) were transferred to HPLC vials. Urine samples were randomly allocated to one of six analytical batches. Each batch contained 1 matrix blank, 1 matrix blank spiked with internal standards, 8-point calibration standards, 3 quality control (QC) samples and 20 urine samples. QC samples were prepared using charcoal stripped human urine from newborns (West Biological Inc., Temecula, CA, USA) with no detectable levels of oestrogen metabolites, spiked with all 14 EMs at a concentration of 2 ng ml⁻¹.

Samples (10 μl) were then analysed by HPLC-electrospray ionisation/MS-MS using an Agilent 1100 HPLC coupled to an SCIEX QTRAP 6500 mass spectrometer (AB Sciex LLC, Framingham, MA, USA) running in multiple reaction monitoring (MRM) mode. Chromatographic separation was carried out on a Phenomenex Synergi Hydro-RP 4 μm × 150 mm × 2.0 mm column, at 40 °C. The solvent gradient used was 35%A (99.8% H2O: 0.2% CHOHO) to 85%B (99.8% MeOH: 0.2% CHOHO) over 60 min. Solvent B was held at 85% for 4 min, then the solvent returned to 35% A for 10 min equilibration before the next injection. The solvent flow rate was 250 μl min⁻¹. The ESI source (type: Ion Drive Turbo V) parameters were set to the following:

TEM 500°C, Curtain Gas 45 psi, GS1 50 psi, GS2 60 psi and MS parameters were CAD gas Medium, DP 80, EP 10, CE 45 and CXP 5. A scheduled detection method was used and the MRM detection window was 120 s with a target scan time of 1 s. Transitions and retention times are listed in Supplementary Table 1.

Analyt 1.6.2 software (AB Sciex LLC) was used for quantification of the EMs. Peak quantifications were carried out using d-EM internal standards and constructing matrix matched (charcoal stripped human urine) eight-point calibration curves for each of the six analytical batches. The calibration curves were evaluated by plotting the peak area ratios of dansyl-EM/d-dansyl-EM against concentration (ng ml⁻¹) of EMs in the standard and using linear regression with 1/X weighting to fit the data. Using this linear function, the amounts of EMs in the urine sample were interpolated.

The intra- and inter-batch coefficients of variation (CVs), evaluated from three QC samples per analytical batch, in six independent consecutive batches (N=18 QC samples) ranged from 6% to 10% (intra-batch CV) and 6% to 14% (inter-batch CV; Supplementary Table 2). The two highest inter-batch CVs were for 4-methoxyestrone (14%) and 4-methoxyestradiol (13%), the two oestrogen metabolites that had the lowest concentrations and which were subsequently excluded from the analysis. The LLOQ was estimated as 80 pg ml⁻¹, where the intra- and inter-batch precision of all the EMs were consistently <10% and the intra- and inter batch accuracies were between 97 and 105%. The LOD for the entire assay (all 14 EMs) was estimated to be 8 pg ml⁻¹.

Statistical analysis. EMs were converted from ng ml⁻¹ of urine to pg mg⁻¹ creatinine using the molecular weight of the unconjugated form of each of the EMs and the amount of creatinine (measured in mg ml⁻¹) for each of the samples. This allowed us to create pathway variables as described by Faupel-Badger et al (2010). Where both of the two samples per woman were measured successfully, we used the mean of the two measurements; where one of two measurements from a woman was missing, we used the one available measurement; where both measurements were missing, we excluded the woman from the analysis of this EM. For the majority of EMs, there were no missing values. For four EMs (2-hydroxyestrone (2-OHE1), 2-methoxyestradiol (2-MeOE2), 2-hydroxyestrone-3-methyl ether (3-MeOE1) and 16-ketoestradiol (16-ketoE2)), there were <10% missing values (Supplementary Table 3). For two EMs (4-methoxyestrone (4-MeOE1) and 4-methoxyestradiol (4-MeOE2)), levels were undetectable in the majority of samples (92 (76.7%) and 107 (89.2%) for 4-MeOE1 and 4-MeOE2, respectively). These two EMs were excluded from further analysis. For the 12 EMs that we were able to measure, we estimated the within-woman variation based on the two sequential samples per woman; intra-class correlation coefficients for individual EMs and grouped EMs are shown in Supplementary Table 4.

For individual and pathway EMs, we calculated geometric means and 95% confidence intervals (CIs) on the natural logarithm scale and exponentiated the values back to the original scale. Linear regression models of the log₂-transformed EMs were used to estimate per cent differences between CYP3A7*1C carriers and non-carriers for individual and pathway EMs. We carried out unadjusted analyses and analyses that were adjusted for measurement batch (1–6), body mass index (BMI; quartiles), age at first full-term pregnancy (quartiles) and parity (0, 1–2, >2). Adjustment for these covariates did not alter effect sizes substantially and, therefore, unadjusted results are presented. We used t-tests of the linear regression coefficient to estimate P-values. We applied a Bonferroni correction to establish a statistical significance level of P<0.003, based on our measuring of 14 individual EMs and 5 grouped EMs. Statistical analyses were carried out using

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R (version 3.2.3; http://cran.r-project.org). All reported P-values are two-sided.

RESULTS

Characteristics of the 60 women who were included in this analysis are presented in Table 1; there were no differences between CYP3A7*1C carriers and non-carriers. Levels of EMs in CYP3A7*1C carriers and non-carriers are shown in Figure 2 (full details of individual EMs, grouped EMs and ratios of EMs are in Supplementary Table 3). The predominant oestrogen/EMs were estriol (E₃), E₁ and 2-hydroxyestrone (2-OHE₁) with geometric mean concentrations in non-carriers of 63.6, 28.6 and 21.8 pmol mg⁻¹ creatinine, respectively. The least abundant EMs were the methylated 2-catechol EMs, 2-methoxyestradiol (2-MeOE₂) and 2-Hydroxyestrone-3-methyl ether (3-MeOE₁) and 3-MeOE₂ with geometric mean concentrations of 1.6, 0.6 and 0.4 pmol mg⁻¹ creatinine, respectively.

Comparing CYP3A7*1C carriers with non-carriers, levels of the parent oestrogen E₁ were 45.3% lower in CYP3A7*1C carriers (P = 0.0005, Table 2). For the two catechol EMs (2-OHE₁ and 2-OHE₂) and the methylated two catechol EMs (2-MeOE₁ and 2-MeOE₂), the reduction in urinary levels was more extreme; compared with non-carriers, levels in CYP3A7*1C carriers were −78.3% (P = 1.1 × 10⁻¹²), −67.9% (P = 2.7 × 10⁻⁷), −81.2% (P = 1.9 × 10⁻¹²) and −62.8% (P = 0.0009) for 2-OHE₁, 2-OHE₂, 2-MeOE₁ and 2-MeOE₂, respectively (Table 2). By contrast, levels of the 16-pathway EMs were slightly higher in CYP3A7*1C carriers, that is, +25.5% (P = 0.11), +91.6% (P = 0.007) and +160.1% (P = 0.002) higher for E₃, 16α-hydroxyestrone (16α-OHE₁) and 17-epiE₃, respectively (Table 2). Adjusting for measurement batch, BMI, age at first full-term pregnancy and parity did not alter these results substantially (Supplementary Table 5).

Table 1. Characteristics of the 30 CYP3A7*1C carriers and 30 CYP3A7*1C non-carriers included in this analysis

|                      | CYP3A7*1C carriers | CYP3A7*1C non-carriers | P-value |
|----------------------|------------------|------------------------|---------|
| Age at urine collection | 44.1 (14–53)    | 45.5 (27–58)           | 0.38    |
| BMI at urine collection | 24.0 (19.2–39.9) | 25.5 (18.3–39.0)       | 0.64    |
| Parity               |                  |                        |         |
| Nulliparous          |                  |                        |         |
| 1–2 Children         | 10 (33.3)        | 6 (20.0)               |        |
| >2 Children          | 17 (56.7)        | 15 (50.0)              |        |
| Age at first full-term pregnancy | 26.3 (20–31) | 27.2 (17–32) | 0.39 |

Abbreviation: BMI—body mass index.

DISCUSSION

To our knowledge, comprehensive data on urinary EMs in premenopausal women, measured using LC-MS, have been previously reported in three studies (Eliassen et al, 2009, 2012; Faupel-Badger et al, 2010; Maskarinec et al, 2012). The first of these was the Nurses’ Health Study II, a prospective study of North American registered nurses aged 25–42 years at recruitment (Eliassen et al, 2009, 2012). The second was a population-based study of incident breast cancer among women of Asian ancestry living in California and Hawaii (Faupel-Badger et al, 2010) and the third was a randomised trial of the effect of consuming soy foods on hormonal outcomes, conducted in women of Caucasian, Native Hawaiian and Asian ancestry, and living in Hawaii (Maskarinec et al, 2012).

Comparing absolute levels of EMs in our study with those reported by these other studies is not straightforward. Levels of individual EMs and all EMs combined may differ between ethnicities (Maskarinec et al, 2012) and women from several different ethnicities have been included in the reports to date (Asian, Faupel-Badger et al (2010); African-American, Asian, Hispanic and Caucasian, Eliassen et al (2012); Caucasian, Native Hawaiian and Asian, Maskarinec et al (2012)). In addition, the 30 non-carriers that we analysed may not be representative of the general British population, as they were selected on genotype (with a MAF of 4%, we would expect 2.2 CYP3A7*1C carriers in a population-based sample of 30 women) and 6 (20%) were first-degree relatives of breast cancer cases, suggesting that they may be
at higher risk than the general population. Our analysis is based on the average of two periovulatory samples, Faupel-Badger et al (2010) analysed both non-luteal phase and luteal phase samples, whereas the studies reported by Eliassen et al (2009, 2012) focussed on luteal phase samples. There is, however, no evidence that the 2-OHE1:16z-OHE1 ratio differs according to the phase of the menstrual cycle (Faupel-Badger et al, 2010). Comparing this ratio across studies, the 2-OHE1:16z-OHE1 ratios reported by Faupel-Badger et al (2010) (2.2 and 2.1 in luteal phase and non-luteal phase samples, respectively) and by Eliassen et al (2012) (4.35) but somewhat lower than the ratios reported by Maskarinec et al (2012) (8.4 and 13.0 in Asian and non-Asians, respectively).

We have previously demonstrated that the CYP3A7*1C allele is associated with lower levels of urinary E2, which are expected to increase risk. Our previous analyses demonstrate that the 2-OHE1:16z-OHE1 ratio differs according to the phase of the menstrual cycle (Faupel-Badger et al, 2010). Comparing this ratio across studies, the 2-OHE1:16z-OHE1 ratios reported by Faupel-Badger et al (2010) (2.2 and 2.1 in luteal phase and non-luteal phase samples, respectively) and by Eliassen et al (2012) (4.35) but somewhat lower than the ratios reported by Maskarinec et al (2012) (8.4 and 13.0 in Asian and non-Asians, respectively).

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consistent with an unfavourable (reduced) 2-hydroxylation:16α-
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Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 27(4): 383–391.

Lee AJ, Conney AH, Zhu BT (2003) Human cytochrome P450 3A7 has a distinct high catalytic activity for the 16alpha-hydroxylation of estrone but not 17beta-estradiol. Cancer Res 63(19): 6532–6536.

Maskarinec G, Heak S, Morimoto Y, Custer L, Franke AA (2012) The relation of urinary estrogen metabolites with mammographic densities in premenopausal women. Cancer Epidemiol 36(5): e310–e316.

Samavat H, Kurzer MS (2015) Estrogen metabolism and breast cancer. Cancer Lett 356(2 Pt A): 231–243.

Tsuchiya Y, Nakajima M, Yokoi T (2005) Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett 227(2): 115–124.

Walker K, Fletcher O, Johnson N, Coupland B, McCormack VA, Folkerd E, Gibson L, Hillier SG, Holly JM, Moss S, Dowsett M, Peto J, dos Santos Silva I (2009) Premenopausal mammographic density in relation to cyclic variations in endogenous sex hormone levels, prolactin, and insulin-like growth factors. Cancer Res 69(16): 6490–6499.

Williams JA, Ring BJ, Cantrell VE, Jones DR, Eckstein J, Ruterbories K, Hamman MA, Hall SD, Wrighton SA (2002) Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. Drug Metab Dispos 30(8): 883–891.

Xu X, Keefer LK, Ziegler RG, Veenstra TD (2007) A liquid chromatography-mass spectrometry method for the quantitative analysis of urinary endogenous estrogen metabolites. Nat Protoc 2(6): 1350–1355.

Xu X, Veenstra TD, Fox SD, Roman JM, Issaq HJ, Falk R, Saavedra JE, Keefer LK, Ziegler RG (2005) Measuring fifteen endogenous estrogens simultaneously in human urine by high-performance liquid chromatography-mass spectrometry, Anal Chem 77(20): 6646–6654.

Ziegler RG, Fuhrman BJ, Moore SC, Matthews CE (2015) Epidemiologic studies of estrogen metabolism and breast cancer. Steroids 99(Pt A): 67–75.

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