Genetic variation at CYP3A is associated with age at menarche and breast cancer risk: a case-control study

Johnson, Nichola; Dudbridge, Frank; Orr, Nick; Gibson, Lorna; Jones, Michael E.; Schoemaker, Minouk J.; Folkerd, Elizabeth J.; Haynes, Ben P.; Hopper, John L.; Southey, Melissa C.; Dite, Gillian S.; Apicella, Carmel; Schmidt, Marjanka K.; Broeks, Annegien; Van't Veer, Laura J.; Atsma, Femke; Muir, Kenneth; Lophatananon, Artitaya; Fasching, Peter A.; Beckmann, Matthias W.; Ekici, Arif B.; Renner, Stefan P.; Sawyer, Elinor; Tomlinson, Ian; Kerin, Michael; Miller, Nicola; Burwinkel, Barbara; Marme, Frederik; Schneeweiss, Andreas; Sohn, Christof; Guenel, Pascal; Truong, Therese; Cordin, Emilie; Menegaux, Florence; Bojesen, Stig E.; Nordestgaard, Borge G.; Flyger, Henrik; Milne, Roger; Zamora, M. Pilar; Arias Perez, Jose Ignacio; Benitez, Javier; Bernstein, Leslie; Anton-Culver, Hoda; Ziegas, Argyrios; Dur, Christina Clarke; Brenner, Hermann; Mueller, Heiko; Arndt, Volker; Dieffenbach, Aida Karina; Meindl, Alfons

Published in:
Breast Cancer Research

DOI:
10.1186/bcr3662

2014

Link to publication

Citation for published version (APA):
Genetic variation at CYP3A is associated with age at menarche and breast cancer risk: a case-control study


* Correspondence: nichola.johnson@icr.ac.uk
† Equal contributors
1 Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK
2 Division of Breast Cancer Research, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK
Full list of author information is available at the end of the article

© 2014 Johnson et al; licensee BioMed Central Ltd. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Introduction

Family history is a well-established risk factor for breast cancer. First-degree relatives of women with breast cancer have an approximately twofold increased risk of developing the disease relative to the general population [1]. Twin studies are consistent with this familial clustering having, at least in part, a genetic origin [2,3]. Mutations in high-risk susceptibility genes (mainly BRCA1 and BRCA2) explain most large multiple-case families, but account for only 15 to 20% of the excess familial risk [4]. Genome-wide association studies [5,6] have identified more than 70 common variants that are associated with breast cancer susceptibility but they account for only another approximately 15% of the excess familial risk. The so-called ‘missing heritability’ may be explained by common variants with very small effects and/or by rarer variants with larger effects, neither of which can be identified by current genome-wide association studies. A statistically efficient alternative is to increase power by trying to identify variants associated with known quantitative phenotypic markers of susceptibility to breast cancer [7], and then to test them for association with breast cancer risk. This approach might also improve our understanding of the biological mechanisms involved in breast cancer pathogenesis.

Endogenous sex hormones are well-established risk factors for breast cancer in postmenopausal women [8]; the evidence in premenopausal women is less consistent, with some, but not all, studies suggesting an association between higher circulating levels of estrogens and increased breast cancer risk [9-17]. Genetic factors influence the levels of endogenous sex hormones [18] and therefore single nucleotide polymorphisms (SNPs) in genes regulating these hormonal pathways are good candidates for being breast cancer predisposition variants. We have previously studied 642 SNPs tagging 42 genes that might influence sex hormone levels in 729 healthy premenopausal women of European ancestry in relation to cyclic variations in oestrogen levels during the menstrual cycle. We found that the minor allele of rs10273424, which maps 50 kb 3’ to CYP3A5, was associated with a reduction of 22% (95% confidence interval (CI) = −28%, −15%; $P = 10^{-8}$) in levels of urinary oestrone glucuronide, a metabolite that is highly correlated with serum oestradiol levels [19]. Analysis of 10,551 breast cancer cases and 17,535 controls of European ancestry demonstrated that the minor allele of rs10235235, a proxy for rs10273424 ($r^2 = 1.0$), was also associated with a weak reduction in
breast cancer risk but only in women aged 50 years or younger at diagnosis (odds ratio (OR) = 0.91, 95% CI = 0.83, 0.99; \( P = 0.03 \)) \[19\].

The aim of the present study was to further investigate an association between rs10235235 and breast cancer risk using a much larger set of subjects – the Breast Cancer Association Consortium (BCAC) – comprising data from 49 additional studies, and to assess whether there was evidence of effect modification by age at diagnosis, ethnicity, age at menarche or tumour characteristics.

Materials and methods
Sample selection
Samples for the case–control analyses were drawn from 52 studies participating in the BCAC: 41 studies from populations of predominantly European ancestry, nine studies of Asian ancestry and two studies of African-American ancestry. The majority were population-based or hospital-based case–control studies, but some studies were nested in cohorts, selected samples by age, oversampled for cases with a family history or selected samples on the basis of tumour characteristics (Table S1 in Additional file 1). Studies provided ~2% of samples in duplicate for quality control purposes (see below). Study subjects were recruited on protocols approved by the Institutional Review Boards at each participating institution, and all subjects provided written informed consent (Additional file 2).

Genotyping and post-genotyping quality control
Genotyping for rs10235235 was carried out as part of a collaboration between the BCAC and three other consortia (the Collaborative Oncological Gene-environment Study (COGS)). Full details of SNP selection, array design, genotyping and post-genotyping quality control have been published \[5\]. Briefly, three categories of SNPs were chosen for inclusion in the array: SNPs selected on the basis of pooled genome-wide association study data; SNPs selected for the fine-mapping of published risk loci; and candidate SNPs selected on the basis of previous analyses or specific hypotheses. rs10235235 was a candidate SNP selected on the basis of our previous analyses \[19\].

For the COGS project overall, genotyping of 211,155 SNPs in 114,225 samples was conducted using a custom Illumina Infinium array (iCOGS; Illumina, San Diego, CA, USA) in four centres. Genotypes were called using Illumina’s proprietary GenCall algorithm. Standard quality control measures were applied across all SNPs and all samples genotyped as part of the COGS project. Samples were excluded for any of the following reasons: genotypically not female XX (XY, XXY or XO, \( n = 298 \)); overall call rate <95% (\( n = 1,656 \)); low or high heterozygosity (\( P < 10^{-6} \), separately for individuals of European, Asian and African-American ancestry, \( n = 670 \)); individuals not concordant with previous genotyping within the BCAC (\( n = 702 \)); individuals where genotypes for the duplicate sample appeared to be from a different individual (\( n = 42 \)); cryptic duplicates within studies where the phenotypic data indicated that the individuals were different, or between studies where genotype data indicated samples were duplicates (\( n = 485 \)); first-degree relatives (\( n = 1,981 \)); phenotypic exclusions (\( n = 527 \)); or concordant replicates (\( n = 2,629 \)).

Ethnic outliers were identified by multidimensional scaling, combining the iCOGS array data with the three Hapmap2 populations, based on a subset of 37,000 uncorrelated markers that passed quality control (including ~1,000 selected as ancestry informative markers). Most studies were predominantly of a single ancestry (European or Asian), and women with >15% minority ancestry, based on the first two components, were excluded (\( n = 1,244 \)). Two studies from Singapore (SGBCC) and Malaysia (MYBRCa; see Table S1 in Additional file 1 for all full study names) contained a substantial fraction of women of mixed European/Asian ancestry (probably of South Asian ancestry). For these studies, no exclusions for ethnic outliers were made, but principal components analysis (see below) was used to adjust for inflation in these studies. Similarly, for the two African-American studies (NBHS and SCCS), no exclusions for ethnic outliers were made.

Principal component analyses were carried out separately for the European, Asian and African-American subgroups, based on a subset of 37,000 uncorrelated SNPs. For the analyses of European subjects, we included the first six principal components as covariates, together with a seventh component derived specific to one study (LMBC) for which there was substantial inflation not accounted for by the components derived from the analysis of all studies. Addition of further principal components did not reduce inflation further. Two principal components were included for the studies conducted in Asian populations and two principal components were included for the African-American studies.

For the main analyses of rs10235235 and breast cancer risk, we excluded women from three studies (BBCS, BIGGS and UKBGS) that were genotyped in the hypothesis-generating study (\( n = 5,452 \)) \[19\] and women with non-invasive cancers (ductal carcinoma in situ/lobular carcinoma in situ, \( n = 2,663 \)) or cancers of uncertain status (\( n = 960 \)). After exclusions there were 47,346 invasive breast cancer case samples and 47,570 control samples from 49 studies (38 from populations of predominantly European ancestry, nine Asian and two African-American) used in the analysis (Tables S1 and S2 in Additional file 1). After quality control exclusions (above) the call rate for rs10235235 was 100% (one no call in 94,916 samples), and for the controls there was no evidence of deviation from
Hardy–Weinberg equilibrium in any of the contributing studies (Table S2 in Additional file 1).

We did not test for an association between rs10235235 and age at menarche in our hypothesis-generating study [19]. Therefore, to maximise our power to detect an association, we included menarche data from BBCS cases (n = 2,508) and controls (n = 1,650) and from UKBGS cases (n = 3,388) and controls (n = 4,081) in this analysis. Age at menarche was not available for samples from BIGGS. Full details of genotyping of rs10235235 in BBCS and UKBGS samples have been published previously [19]. Briefly, genotyping was carried out using competitive allele-specific polymerase chain reaction KASPar chemistry (KBiosciences Ltd, Hoddesdon, Hertfordshire, UK). Call rates were 98.0% (BBCS) and 96.6% (UKBGS); there was no evidence for deviation from Hardy–Weinberg equilibrium (P = 0.29 (BBCS); P = 0.92 (UKBGS)), and the duplicate concordance based on a 1% (BBCS) and 5% (UKBGS) random sample of duplicates was 100% for both studies.

Statistical analysis
We estimated per-allele and genotypic log odds ratios (ORs) for the European, Asian and African-American subgroups separately using logistic regression, adjusted for principal components and study [5]. To test for departure from a multiplicative model we compared multiplicative and unconstrained models using a one degree of freedom likelihood ratio test. Heterogeneity in ORs between studies within each subgroup (European, Asian and African-American), and between subgroups, was assessed using the Cochrane Q statistic and quantified using the I² measure [20].

Analyses stratified by oestrogen receptor status (+/–), progesterone receptor status (+/–), morphology (ductal or lobular), grade (1,2,3), lymph node involvement (+/–) or age at diagnosis (≤50 and >50 years) were restricted to studies of European ancestry due to the small number of studies of Asian and African-American ancestry. In addition, studies were excluded if they had selected cases on the basis of the stratifying variable, or had collected data on that variable for less than 5% of cases or less on the basis of the stratifying variable, or had collected data on age at diagnosis (grouped as ≤11, 12, 13, 14 and ≥15 years) with and without an interaction term(s). We considered four models: no interaction (zero interaction terms); assuming a linear interaction between genotype and menarche group (one interaction term); assuming a linear interaction between genotype and menarche group but allowing the linear term to differ between women who were heterozygous and those who were homozygous for the rare allele (two interaction terms); and one interaction term for each possible genotype/menarche group combination (eight interaction terms). Nested models were compared using likelihood ratio tests. All statistical analyses were performed using STATA version 11.0 (StataCorp, College Station, TX, USA). All P values reported are two-sided.

Results
The case–control analysis comprised genotype data for 47,346 invasive breast cancer cases and 47,569 controls from 49 studies, including 80,518 (84.8%) subjects of self-reported European ancestry, 12,419 (13.1%) of self-reported Asian ancestry and 1,978 (2.1%) of self-reported African-American ancestry. The mean (± standard deviation) age at diagnosis was 56.1 (± 11.6) years for European cases, 51.1 (± 10.5) years for Asian cases and 53.1 (± 10.7) years for African-American cases. There were ethnic differences in the estimated minor allele frequency (MAF) of rs10235235 (Q = 7317.1, two degrees of freedom; P for heterogeneity (Phet) = 0). The overall MAF for European control women was 0.089 (95% CI = 0.087, 0.091), but with strong evidence of between-study heterogeneity (Phet = 1 x 10^{-22}) that was accounted for by the three Finnish studies (HEBCS, MAF = 0.15; KBCP, MAF = 0.21; and OBCS, MAF = 0.15; Phet = 0.01); no evidence of heterogeneity remained after taking account of these studies (MAF = 0.087 (95% CI = 0.085, 0.089); Phet = 0.23).

Relative to Europeans, the overall MAF was higher for African-Americans (0.213, 95% CI = 0.195, 0.232; Phet = 0.26) but much lower for Asians (0.002; 95% CI = 0.001, 0.002), with strong evidence of between-study heterogeneity for the latter (Phet = 4 x 10^{-14}).
The case–control analysis was consistent with a modest association between rs10235235 and breast cancer risk for women of European ancestry, with an estimated per-allele OR of 0.96 (95% CI = 0.93, 0.99; P for linear trend (P_{trend}) = 0.02). Genotype-specific ORs were 0.98 (95% CI = 0.94, 1.01; P = 0.21) for AG versus AA (Figure 1A) and 0.80 (95% CI = 0.69, 0.93; P = 0.004) for GG versus AA (Figure 1B), with no evidence of between-study heterogeneity for either OR estimate (P_{het} = 0.44, I^2 = 1.9% and P_{het} = 0.76, I^2 = 0.0% for heterozygote and homozygote OR estimates respectively). There was, however, marginally significant evidence that the genotypic OR estimates departed from those expected under a multiplicative model with the inverse association of the GG genotype being more than the square of that of the AG genotype (test for deviation from multiplicative model, P = 0.04).

Data for rs10235235 in women of Asian or African-American ancestry were more limited, with just two African-American studies (1,046 cases and 932 controls) and nine Asian studies (5,795 cases and 6,624 controls). In addition, this SNP was sufficiently rare in Asian populations (MAF = 0.002) that we were unable to estimate a homozygote OR for any Asian study (Table S2 in Additional file 1). There was no clear evidence that this SNP was associated with breast cancer risk for women of Asian ancestry (heterozygote OR = 1.06, 95% CI = 0.76, 1.49) or African-American ancestry (heterozygote and homozygote ORs were OR = 1.09, 95% CI = 0.90, 1.32 and OR = 0.94, 95% CI = 0.62, 1.42 respectively; Figure S1 in Additional file 1). This analysis, however, had low power to detect associations in non-Europeans and these OR estimates were not inconsistent with the magnitude of the observed OR estimates for European women (P_{het} = 0.51).

Stratifying cases by oestrogen receptor (P_{het} = 0.83) or progesterone receptor (P_{het} = 0.19) status, tumour grade (P_{het} = 0.63) or nodal involvement at diagnosis (P_{het} = 0.51) showed no evidence of effect modification (Table 1). There was some evidence of effect modification by morphology (P_het = 0.03). For ductal cancers we estimated a very modest reduction of risk for heterozygotes (OR_{het} = 0.98, 95% CI = 0.93, 1.02; P = 0.30) and a stronger, significant reduction for homozygotes (OR_{hom} = 0.74, 95% CI = 0.61, 0.90; P = 0.003). For lobular cancers there was no such trend (OR_{het} = 1.07, 95% CI = 0.98, 1.17; P = 0.14 and OR_{hom} = 0.91, 95% CI = 0.64, 1.27; P = 0.57).

The SNP rs10235235 maps to a locus (CYP3A) that has been considered an a priori candidate for involvement in determining age at menopause and age at menarche [21,22]. Stratifying cases by age at diagnosis (≤50

**Figure 1** Association of rs10235235 with breast cancer risk for women of European ancestry. Forest plots of the association of the rs10235235 AG (heterozygote) genotype (A) and GG (homozygote) genotype (B) with breast cancer risk for women of European ancestry. Horizontal lines, 95% confidence intervals (CIs); square boxes, study-specific fixed-effects estimates; diamond, combined, fixed-effects estimate of the odds ratio (OR) and 95% CI. Vertical line, null effect (OR = 1.0); dashed vertical line, estimated heterozygote OR (A) and estimated homozygote OR (B). Homozygote ORs for six studies (CTS, DEMOKRITOS, KConFab/AOCS, NBCS, NBHS and RPCI) could not be estimated because there were no GG homozygotes among cases or among controls in each of these studies (see Table S2 in Additional file 1).
or >50 years) as a proxy for menopausal status at diagnosis showed no evidence of effect modification ($P_{\text{het}} = 0.89$; Table 2), and excluding cases who were diagnosed between age 46 and 55 as potentially perimenopausal did not alter this result ($P_{\text{het}} = 0.28$). Data on age at menarche were available for 21,736 cases and 22,686 controls (Table S4 in Additional file 1); to increase the power of the analysis we included additional data from BBCS and UKBGS (5,737 cases, 5,572 controls; Table S4 in Additional file 1) [19]. There was a 1.5% (95% CI = 0.5%, 2.7%; $P_{\text{het}} = 0.004$) reduction in breast cancer risk associated with each additional year’s increase in age at menarche. Mean age at menarche was positively associated with number of copies of the minor allele of rs10235235 for controls ($P_{\text{trend}} = 0.005$; Table 3) but not for cases ($P_{\text{trend}} = 0.97$; Table 3). Consequently, there was an inverse trend in the magnitude of the heterozygote and homozygote breast cancer ORs with mean age at menarche ($P_{\text{het}} = 0.02$; Table 4); being a carrier of one or two rare alleles of rs10235235 was associated with an estimated 16% (OR$_{\text{het}} = 0.84$, 95% CI = 0.75, 0.94; $P = 0.003$) or 19% (OR$_{\text{hom}} = 0.81$, 95% CI = 0.51, 1.30; $P = 0.39$) ($P_{\text{trend}} = 0.002$) reduction in breast cancer risk for women who had their menarche at ages ≥15 years but there was no evidence of reduction for those with a menarche at age ≤11 years (OR$_{\text{het}} = 1.06$, 95% CI = 0.95, 1.19; $P = 0.30$ and OR$_{\text{hom}} = 1.07$, 95% CI = 0.67, 1.72; $P = 0.78$) ($P_{\text{trend}} = 0.29$). There was no evidence that the inverse trend in the magnitude of ORs with mean age at menarche differed between heterozygous and homozygous carriers ($P = 0.97$) and no evidence that the trend was nonlinear ($P = 0.70$).

### Table 1 Association of rs10235235 with risk of breast cancer for women of European ancestry: stratified analysis

<table>
<thead>
<tr>
<th>Status</th>
<th>Cases</th>
<th>Controls</th>
<th>OR$_{\text{het}}$</th>
<th>95% CI</th>
<th>$P_{\text{1}}$</th>
<th>OR$_{\text{hom}}$</th>
<th>95% CI</th>
<th>$P_{\text{1}}$</th>
<th>$P_{\text{het}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-positive</td>
<td>24,780</td>
<td>38,739</td>
<td>0.99</td>
<td>0.95, 1.03</td>
<td>0.61</td>
<td>0.83</td>
<td>0.70, 0.99</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>ER-negative</td>
<td>5,851</td>
<td>38,739</td>
<td>1.02</td>
<td>0.95, 1.10</td>
<td>0.60</td>
<td>0.60</td>
<td>0.43, 0.86</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>8,339</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>38,970$^a$</td>
<td>38,739</td>
<td>0.99</td>
<td>0.95, 1.03</td>
<td>0.74</td>
<td>0.79</td>
<td>0.67, 0.94</td>
<td>0.006</td>
<td>0.83</td>
</tr>
<tr>
<td>PR status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR-positive</td>
<td>18,497</td>
<td>39,033</td>
<td>0.98</td>
<td>0.93, 1.02</td>
<td>0.32</td>
<td>0.82</td>
<td>0.67, 0.99</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>PR-negative</td>
<td>8,193</td>
<td>39,033</td>
<td>1.02</td>
<td>0.96, 1.09</td>
<td>0.53</td>
<td>0.74</td>
<td>0.56, 0.98</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>12,111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>38,801$^b$</td>
<td>39,033</td>
<td>0.99</td>
<td>0.94, 1.03</td>
<td>0.52</td>
<td>0.80</td>
<td>0.67, 0.95</td>
<td>0.01</td>
<td>0.19</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>22,123</td>
<td>31,803</td>
<td>0.98</td>
<td>0.93, 1.02</td>
<td>0.30</td>
<td>0.74</td>
<td>0.61, 0.90</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Lobular</td>
<td>3,921</td>
<td>31,803</td>
<td>1.07</td>
<td>0.98, 1.17</td>
<td>0.14</td>
<td>0.91</td>
<td>0.64, 1.27</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Other and NK</td>
<td>5,995</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32,039</td>
<td>31,803</td>
<td>0.99</td>
<td>0.95, 1.04</td>
<td>0.64</td>
<td>0.77</td>
<td>0.64, 0.92</td>
<td>0.004</td>
<td>0.03</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>5,944</td>
<td>37,285</td>
<td>0.97</td>
<td>0.90, 1.05</td>
<td>0.46</td>
<td>0.86</td>
<td>0.65, 1.15</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>13,427</td>
<td>37,285</td>
<td>1.00</td>
<td>0.95, 1.06</td>
<td>0.92</td>
<td>0.80</td>
<td>0.63, 0.98</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>8,638</td>
<td>37,285</td>
<td>0.98</td>
<td>0.92, 1.05</td>
<td>0.58</td>
<td>0.61</td>
<td>0.46, 0.82</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>8,769</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>36,778</td>
<td>37,285</td>
<td>0.99</td>
<td>0.95, 1.03</td>
<td>0.56</td>
<td>0.76</td>
<td>0.64, 0.90</td>
<td>0.001</td>
<td>0.63</td>
</tr>
<tr>
<td>Nodal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Node-negative</td>
<td>17,463</td>
<td>37,836</td>
<td>0.98</td>
<td>0.93, 1.03</td>
<td>0.47</td>
<td>0.86</td>
<td>0.71, 1.04</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Node-positive</td>
<td>10,746</td>
<td>37,836</td>
<td>0.98</td>
<td>0.92, 1.04</td>
<td>0.46</td>
<td>0.72</td>
<td>0.57, 0.93</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>9,359</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>37,568</td>
<td>37,836</td>
<td>0.98</td>
<td>0.94, 1.02</td>
<td>0.31</td>
<td>0.81</td>
<td>0.68, 0.96</td>
<td>0.02</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Association of rs10235235 with risk of breast cancer for women of European ancestry stratified by oestrogen receptor (ER) status, progesterone receptor (PR) status, morphology, grade and nodal status. OR$_{\text{het}}$, odds ratio comparing rs10235235 AG genotype versus AA genotype; H0, null hypothesis; NK, not known; OR$_{\text{hom}}$, odds ratio comparing rs10235235 GG genotype versus AA genotype; $P_{\text{1}}$, test of H0 no association between rs10235235 and breast cancer risk; $P_{\text{het}}$, test of H0 no difference between stratum specific estimates for variables with two strata or test of H0 no linear trend in stratum specific estimates for variables with three strata. $^a$Excludes seven studies that selected all ER-negative cases (CTS, DEMOKRITOS, NBCS, NBHS, OSU, RPCI and SKKDFZS) and one study (PBCS) that selected all ER-positive cases. $^b$Excludes seven studies that selected all PR-negative cases (CTS, DEMOKRITOS, NBCS, NBHS, OSU, RPCI and SKKDFZS).
**Table 3 Association of rs10235235 with age at menarche for women of European ancestry by case-control status**

<table>
<thead>
<tr>
<th>rs10235235 genotype</th>
<th>Cases</th>
<th>Age at menarche (years)</th>
<th>( \rho_{\text{trend}} )</th>
<th>Controls</th>
<th>Age at menarche (years)</th>
<th>( \rho_{\text{trend}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>22,954</td>
<td>12.83</td>
<td>23,383</td>
<td>12.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>4,312</td>
<td>12.83</td>
<td>4,627</td>
<td>13.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>207</td>
<td>12.83</td>
<td>248</td>
<td>13.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27,473</td>
<td>12.83</td>
<td>0.97</td>
<td>28,258</td>
<td>12.96</td>
<td>0.005</td>
</tr>
</tbody>
</table>

\( H_0 \) null hypothesis; \( \rho_{\text{trend}} \) test of \( H_0 \) no linear trend in age at menarche according to rs10235235 genotype.

**Table 2 rs10235235 and risk of breast cancer for women of European ancestry by age at diagnosis**

<table>
<thead>
<tr>
<th>Age at diagnosis</th>
<th>Cases(^a)</th>
<th>Controls(^a)</th>
<th>( \text{OR}_{\text{het}} )</th>
<th>( 95% \text{ CI} )</th>
<th>( P_1 )</th>
<th>( \text{OR}_{\text{hom}} )</th>
<th>( 95% \text{ CI} )</th>
<th>( P_1 )</th>
<th>( P_\text{het} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \leq 50 \text{ years} )</td>
<td>11,794</td>
<td>34,988</td>
<td>0.99</td>
<td>0.93, 1.05</td>
<td>0.09</td>
<td>0.68</td>
<td>0.53, 0.86</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>( &gt; 50 \text{ years} )</td>
<td>23,264</td>
<td>34,988</td>
<td>0.97</td>
<td>0.93, 1.02</td>
<td>0.24</td>
<td>0.84</td>
<td>0.70, 1.00</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>554</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35,612</td>
<td>34,988</td>
<td>0.98</td>
<td>0.94, 1.02</td>
<td>0.23</td>
<td>0.79</td>
<td>0.67, 0.92</td>
<td>0.003</td>
<td>0.89</td>
</tr>
</tbody>
</table>

\(^a\)Five studies (ABCF5, MARIE, MEC, MTLGGBCS and SASBAC) that selected all cases on the basis of age at diagnosis (Table S3 in Additional file1) were excluded from this stratified analysis; two small studies (CTS and NBCS) that had no heterozygote or rare homozygote cases in one of the age stratum were also excluded. \( H_0 \), null hypothesis; NK, not known; \( \text{OR}_{\text{het}} \), odds ratio comparing rs10235235 AG genotype versus AA genotype; \( \text{OR}_{\text{hom}} \), odds ratio comparing rs10235235 GG genotype versus AA genotype; \( P_1 \), test of \( H_0 \) no association between rs10235235 and breast cancer risk; \( P_\text{het} \), test of \( H_0 \) no difference between stratum specific estimates.

**Discussion**

This study of more than 47,000 breast cancer cases and 47,000 controls has confirmed that rs10235235, mapping to 7q22.1 (CYP3A), is associated with a reduction in breast cancer risk for women of European ancestry. Previously, our hypothesis-generating study of 10,000 breast cancer cases and 17,000 controls found a per-allele OR estimate of 0.96 (95% CI = 0.90, 1.02; \( P = 0.2 \)), with marginally significant evidence of an inverse association for breast cancer diagnosed age 50 years or younger (OR = 0.91, 95% CI = 0.83, 0.99; \( P = 0.03 \)) but no evidence of an association for breast cancer at later ages (OR = 1.01, 95% CI = 0.93, 1.10; \( P = 0.82 \)) [19]. In this considerably larger study, we found a heterozygote OR estimate of 0.98 (95% CI = 0.94, 1.01; \( P = 0.21 \)) and a homozygote OR estimate of 0.80 (95% CI = 0.69, 0.93; \( P = 0.004 \)) with marginally significant evidence that the inverse association for homozygotes is greater than predicted by a multiplicative model (\( P = 0.04 \)).

To our knowledge, rs10235235 is the first SNP to be associated with both breast cancer risk and age at menarche, consistent with the well-documented association between later age at menarche and a reduction in breast cancer risk [23]. Genome-wide association studies have identified more than 70 breast cancer risk variants [5,6] and more than 30 variants associated with age at menarche [22], none of which map to the CYP3A locus. rs10235235 was originally identified on the basis of a highly significant association with hormone levels, accounting for 4.9% of the variation in premenopausal urinary oestrone glucuronide levels [19]. In this current analysis, rs10235235 accounted for only 0.01% of the variation across controls in age at menarche and we estimate that this SNP explains just 0.01% of the familial excess breast cancer risk. Our data thus illustrate the potential statistical efficiency of studies of intermediate phenotypes in the identification of rarer (MAF < 10%) risk alleles with modest associations. Our analysis shows some inconsistency with a recent genome-wide study of circulating oestradiol, testosterone and sex hormone-binding globulin in postmenopausal women [24]. In that study there was no genome-wide significant association observed with plasma oestradiol levels in either the primary analysis of approximately 1,600 postmenopausal women who were not taking postmenopausal hormones at blood draw or the secondary analysis that included approximately 900 current postmenopausal hormone users. Further studies will be needed to determine whether the lack of an association between CYP3A variants and postmenopausal plasma oestradiol levels reflects a difference in the menopausal status of the study subjects, the hormone/metabolite that was analysed or chance.

One possible explanation for the apparent effect modification of the rs10235235–breast cancer risk association by age at menarche is that this is a function of genotyping a marker SNP rather than the true causal variant. For example, if rs10235235 was perfectly correlated with a causal variant, SNP X, with a MAF substantially lower than that of rs10235235 (\( D' \approx 1.0, r^2 < 1.0 \)), then there would be three types of chromosome in the population: type i, chromosomes carrying the common allele of rs10235235 and the common allele of SNP X; type ii, chromosomes carrying the rare allele of rs10235235 and the common allele of SNP X; and type iii, chromosomes carrying the rare allele of rs10235235 and the rare (protective) allele of SNP X. Only chromosomes carrying the rare allele of rs10235235 and the rare (protective) allele of
SNP X (type iii) would be enriched in controls. Genotyping the marker (rs10235235) rather than the causal variant leads to misclassification. As the causal variant is associated with a protective effect on breast cancer risk, the proportion of chromosomes carrying both the rare allele of the causal variant and the marker (type iii) compared with the common allele of the causal variant and the rare allele of the marker (type ii) will be greater in controls than in cases such that the extent of misclassification will be greater for cases than controls. This will attenuate the association between genotype and age at menarche to a greater extent in cases than in controls creating an apparent effect modification. Fine mapping and functional studies will be required to identify the causal variant and to determine the true relationship between the causal variant, age at menarche and breast cancer risk.

Despite our original finding of a strong association between rs10235235 and hormone levels, we found no evidence that the association between this SNP and breast cancer risk differed by the hormone receptor status of the tumour, and nor did we find any evidence that the association differed by stage, grade or lymph node involvement. There was marginally significant evidence that the association between rs10235235 and breast cancer risk differed between ductal and lobular cancers ($P_{\text{het}} = 0.03$). Given the number of stratified analyses that we carried out (six stratifying variables) and given that there is no biological basis to support an interaction between rs10235235 and morphology, this is probably a chance observation.

In contrast to our earlier study [19], we found no evidence of an interaction with age at diagnosis when we stratified cases by age $\leq/\geq 50$ years, either including or excluding cases diagnosed between age 46 and 55 years as potentially perimenopausal. We used age at diagnosis as a proxy for menopausal status at diagnosis because menopausal status at diagnosis is difficult to determine by questionnaire, especially given the use of hormone replacement therapies; while information on age at diagnosis was available for all but 1.4% ($n = 554$) of cases, information on age at natural menopause was missing for 65.6% ($n = 26,552$) of cases of European ancestry. Similarly, although rs10235235 is a plausible candidate for association with age at menopause, we did not test this due to the limited amount of data on age at natural menopause for controls of European ancestry ($n = 11,294, 28.2\%$) and the difficulty in ascertaining whether treatment for breast cancer had influenced reported age at menopause for cases.

The strengths of our study include the large size of this combined analysis, and the availability of information on tumour characteristics for the majority of cases and on age at menarche for the majority of cases and controls. Limitations include low power of the study to examine an association between genotype and breast cancer risk for non-Europeans.

**Conclusions**

In summary, we have confirmed that rs10235235 is associated with breast cancer, have shown for the first time that rs10235235 is associated with age at menarche in controls and have suggested a potential mechanism for these associations. rs10235235, which maps to the CYP3A locus, probably tags a causal variant that affects expression of one or more CYP3A genes.

### Additional files

**Additional file 1:** Contains Table S1 presenting details of participating BCAC studies; Table S2 presenting rs10235235 genotypes for breast cancer cases and controls from 49 BCAC studies; Table S3 presenting availability of data on age at diagnosis, hormone receptor status, morphology, grade and nodal status for breast cancer cases from 38 European BCAC studies; Table S4 presenting availability of data on age at menarche for breast cancer cases and controls from 40 European BCAC studies; and Figure S1 showing association of the rs10235235-AG genotype with breast cancer risk for women of Asian and African-American ancestry.

**Additional file 2:** Presents details of ethical committees that approved each study.

**Abbreviations**

BCAC: Breast Cancer Association Consortium; CI: confidence interval; COGS: Collaborative Oncological Gene-environment Study; MAF: minor allele frequency; OR: odds ratio; $P_{\text{trend}}$: $P$ value for linear trend; SNP: single nucleotide polymorphism.

---

**Table 4** rs10235235 and risk of breast cancer for women of European ancestry by age at menarche

<table>
<thead>
<tr>
<th>Age at menarche (years)</th>
<th>Cases</th>
<th>Controls</th>
<th>ORhet</th>
<th>95% CI</th>
<th>$P_1$</th>
<th>ORhom</th>
<th>95% CI</th>
<th>$P_1$</th>
<th>$P_{\text{het}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤11</td>
<td>4,818</td>
<td>4,749</td>
<td>1.06</td>
<td>0.95, 1.19</td>
<td>0.30</td>
<td>1.07</td>
<td>0.67, 1.72</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5,655</td>
<td>5,720</td>
<td>0.92</td>
<td>0.83, 1.02</td>
<td>0.10</td>
<td>0.83</td>
<td>0.54, 1.28</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>7,308</td>
<td>7,379</td>
<td>0.93</td>
<td>0.85, 1.02</td>
<td>0.11</td>
<td>0.77</td>
<td>0.54, 1.09</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>5,307</td>
<td>5,743</td>
<td>0.96</td>
<td>0.86, 1.06</td>
<td>0.42</td>
<td>0.69</td>
<td>0.45, 1.06</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>≥15</td>
<td>4,385</td>
<td>4,667</td>
<td>0.84</td>
<td>0.75, 0.94</td>
<td>0.003</td>
<td>0.81</td>
<td>0.51, 1.30</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27,473</td>
<td>28,258</td>
<td>0.94</td>
<td>0.90, 0.98</td>
<td>0.007</td>
<td>0.81</td>
<td>0.67, 0.98</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

$H_0$, null hypothesis; ORhet, odds ratio comparing rs10235235 AG genotype versus AA genotype; ORhom, odds ratio comparing rs10235235 GG genotype versus AA genotype; $P_1$, test of $H_0$ no association between rs10235235 and breast cancer risk; $P_{\text{warr}}$, test of $H_0$ no linear trend in stratum specific estimates.
Competing interests

The authors state that they have no competing interests.

Authors' contributions

OF, FD and NO performed the statistical analyses. OF, IdSS and NJ drafted the manuscript. NJ, FD, NO, LG, ME, MIS, EIF, BP, MG-C, MD0, AA, AJS, JP, IdSS and OF comprised the writing group that was responsible for the interpretation of the results and for critically reviewing the manuscript. AC, AJ, AHW, AMa, BBu, CYS, DL, ES, GC-T, HN, HBre, HbLa, ILa, IC-JC-JYC, JLH, LbA, MBK, MHi, PAF, PR, FW, SEB, TD, MKs and UE also significantly contributed to the interpretation of the results. OF, IdSS, NJ, LP, JG, DFE, MB and JW knew the content of the original design of the study and participated in subject recruitment and in acquisition of data. Jben, AG-H, RM, DCT, DF, VB, CL, JD, JS and KMw knew the genotyping and/or data analysis. FD, NO, MEI, MIS, EIF, BP, JH, JCS, MGD, CA, MKs, AB, LVM, FA, KMw, AlO, PAF, MBW, ABE, SPR, ES, IT, MK, NM, BBu, FMa, AS, CS, PG, TT, EC, FMe, SEB, BGF, HM, RM, MP, JAP, JY, Jben, LbAe, HCA, AC, CCD, Hbre, Hmu, VA, AKD, Ame, Jh, CRKs, HbLa, CJ-YD, The GENICA Network, HN, TAM, KA, CB, HMa, TD, NvB, NA, ALu, AMa, VK-VMK, JHm, GC-T, JY, CBee, KConFab Investigators, Australian Ovarian Cancer Study Group, AHw, DvDb, C-Ge, DL, DS, PN, H-IC-JC, AR, SN, DF-J, JR, PR, PP, BBu, BPv, FJc, JED, XW, ZT, VSp, CGG, GS, LbA, CB, CH, JS, MGs, FL, MDu, PT, ST, CHY, SYF, BIC, VNK, GGA, A-LB-D, WZ, RW, KP, A-JV, MG, ILa, JAK, GG, AMM, PF, JD, SJc, LJS, MES, PH, NS, MHO, RA, HAOD, MTL, JU-I, ACwB, MVWR, SSC, WB, LBS, PDPP, AMDs, MS, Dk, D-YN, SKP, J-YC, MHa, HM, WYll, AT, UH, AF, TR, Hlu, Ju, A-JU, KL-B, KD, SSA, VG, PB, JM, SSJ, AET, CV, DY, CYS, J-YC, CH, SF, M-HF, AG-N, DCT, DF, VB, CL, JD, KM, MKb, JW, DFE, MG-C, MD0, AA and AUS made substantial contributions in recruiting subjects and acquiring data, and in critically reviewing the manuscript. All authors take responsibility for the work and read and approved the final version of the manuscript.

Acknowledgements

The authors thank all of the individuals who took part in these studies and all of the researchers, clinicians, technicians and administrative staff who have enabled this work to be carried out.

ABCFS would like to thank Maggie Angelakos, Judd Maskell and Gillian Dite. ABCS would like to thank Ellen van der Schoot and Sanquin Amsterdam. ABCS would like to thank Maggie Angelakos, Judi Maskell and Gillian Dite. ABCFS would like to thank Maggie Angelakos, Judd Maskell and Gillian Dite.

AC, AJ, AHW, AMa, BBu, CYS, DL, ES, GC-T, HN, HBre, HbLa, ILa, IC-JC-JYC, JLH, LbA, MBK, MHi, PAF, PR, FW, SEB, TD, MKs and UE also significantly contributed to the interpretation of the results. OF, IdSS, NJ, LP, JG, DFE, MB and JW knew the content of the original design of the study and participated in subject recruitment and in acquisition of data. Jben, AG-H, RM, DCT, DF, VB, CL, JD, JS and KMw knew the genotyping and/or data analysis. FD, NO, MEI, MIS, EIF, BP, JH, JCS, MGD, CA, MKs, AB, LVM, FA, KMw, AlO, PAF, MBW, ABE, SPR, ES, IT, MK, NM, BBu, FMa, AS, CS, PG, TT, EC, FMe, SEB, BGF, HM, RM, MP, JAP, JY, Jben, LbAe, HCA, AC, CCD, Hbre, Hmu, VA, AKD, Ame, Jh, CRKs, HbLa, CJ-YD, The GENICA Network, HN, TAM, KA, CB, HMa, TD, NvB, NA, ALu, AMa, VK-VMK, JHm, GC-T, JY, CBee, KConFab Investigators, Australian Ovarian Cancer Study Group, AHw, DvDb, C-Ge, DL, DS, PN, H-IC-JC, AR, SN, DF-J, JR, PR, PP, BBu, BPv, FJc, JED, XW, ZT, VSp, CGG, GS, LbA, CB, CH, JS, MGs, FL, MDu, PT, ST, CHY, SYF, BIC, VNK, GGA, A-LB-D, WZ, RW, KP, A-JV, MG, ILa, JAK, GG, AMM, PF, JD, SJc, LJS, MES, PH, NS, MHO, RA, HAOD, MTL, JU-I, ACwB, MVWR, SSC, WB, LBS, PDPP, AMDs, MS, Dk, D-YN, SKP, J-YC, MHa, HM, WYll, AT, UH, AF, TR, Hlu, Ju, A-JU, KL-B, KD, SSA, VG, PB, JM, SSJ, AET, CV, DY, CYS, J-YC, CH, SF, M-HF, AG-N, DCT, DF, VB, CL, JD, KM, MKb, JW, DFE, MG-C, MD0, AA and AUS made substantial contributions in recruiting subjects and acquiring data, and in critically reviewing the manuscript. All authors take responsibility for the work and read and approved the final version of the manuscript.

Consortia members

The GENICA network: Dr Margarette Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University of Tubingen, Germany (Christina Justenzhoven, Hiltrud Brauch); Department of Internal Medicine, Evangelische Kliniken Bonn ggGmbH, Johanniter Krankenhaus, Bonn, Germany (Yon-Di-Chun Ko, Christian BaiSchi); Institute of Pathology, University of Bonn, Germany (Hans-Peter Fischer); Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany (Ute Harnam); Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (lPA), Bochum, Germany (Thomas Bruening, Beate Pech, Sylvia Rabstein, Anne Spickenheuer); and Institute for Occupational Medicine and Maritime Medicine, University Medical Center Hamburg-Eppendorf, Germany (Volk Ter Haar). KConFab Investigators: David Amor, Lesley Andrews, Yoland Antill, Shane Armitage, Rosemary Baleen, Agnes Banker, Patti Bastick, John Beilby, Barbara Bennett, Ian Bennett, Anneke Blackburn, Michael Bogwitz, Meagan Brennan, Melissa Brown, Michael Buckley, Matthew Burgess, Jo Burke, Phyllis Butow, Ian Campbell, Alice Christian, Georgia Chenexiev-Trench, Christine Connell, Alison Colley, Dick Cotton, Bronwyn Gilling, Margaret Cummins, Sarah-Jane Dawson, Anna DeFazio, Martin Delatycki, Rebecca Dickson, Alexander Dobrovic, Tracy Dudding, Ted Edkins, Stacey Edwards, Gelareh Farshid, Susan Fawcett, Georgina Fenton, Michael Field, James Flanagan, Peter Fong, John Forbes, Stephen Fox, Juliet French, Clara Gaff, Mac Gardner, Mike Gattas, Graham Giles, Grant Glatt, Blair Greening, Scott Grist, Eric Haan, Marion Harris, Stewart Hart, Nick Hayward, Sue Healey, Louise Heiniger, John Hopper, Clare Hunt, Paul James, Mark Jenkins, Rick Kefford, Rodney Scott, Adrienne Sexton, Raghwa Sharma, Andrew Shelling, Peter Simpson, Melissa Southey, Amanda Spurdle, Graeme Suthers, Pamela Syes, Jessica Taylor, Elia Thompson, Heather Thorne, Sharron Trinh-Tran, Andrew Trainer, Bob Watson, Logan Walker, Paul Waring, Robin Ward, Bev Warner, Rachael Williams, Ingrid Winship, Mary Ann Young (Peter MacCallum Cancer Center, Melbourne, Australia). The Australian Ovarian Cancer Study Group: David D Bowtell, Adele C Green, Georgia Chenexiev-Trench, Anna DeFazio, Dorota Gertig, Penelope M Webb (Peter MacCallum Cancer Center, Melbourne, Australia).
Financial support

Part of this work was supported by the European Community’s Seventh Framework Programme under grant agreement number 223175 (grant number HEALTH-F2-2009-223175) (COGS). This work was partly supported by the Canadian Institutes of Health Research for the ‘CIHR Team in Familial Risks of Breast Cancer’ program (U5, DF6), and the Ministry of Economic Development, Innovation and Export Trade of Quebec – grant number PSR-SIRI-701 (US, DFE, PH).

The ABCFS and OFBCR work was supported by the United States National Cancer Institute, National Institutes of Health (NIH) under RFA-CA-06-503 and through cooperative agreements with members of the Breast Cancer Family Registry (BCFR) and Principal Investigators, including Cancer Care Ontario (U01 CA69467), Northern California Cancer Center (U01 CA69417) and University of Melbourne (U01 CA69638). Samples from the NC-BCFR were processed and distributed by the Coriell Institute for Medical Research. The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the BCFR, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government or the BCFR. ABCFS was also supported by the National Health and Medical Research Council of Australia, the New South Wales Cancer Council, the Victorian Health Promotion Foundation (Australia) and the Victorian Breast Cancer Research Consortium. JLI is a National Health and Medical Research Council (NHMRC) Australia Fellow and a Victorian Breast Cancer Research Consortium Group Leader. MCS is a NHMRC Senior Research Fellow and a Victorian Breast Cancer Research Consortium Group Leader. The ABCS study was supported by the Dutch Cancer Society (grants 9N1 2001-2413 and 2007-3509) and the Dutch National Genomics Initiative. The ACPS study is funded by the Breast Cancer Research Trust, UK. The work of the BBCS was partly funded by ELAN-Fond of the University Hospital of Erlangen. BBCS is funded by Cancer Research UK and Breast Cancer Research Trust, UK, and acknowledges NHS funding to the NIHR Biomedical Research Centre and the National Cancer Research Network. BCAC is funded by CR-UK (CI287/A10118 and CI287/A1014). Meetings of the BCAC have been funded by the European Union COST programme (BM0606). DFE is a Principal Research Fellow of CR-UK. ES (BIGGS) is supported by the Academy of Finland (132473), Helsinki University Central Hospital Research Foundation, the Sigrid Juselius Foundation, the Finnish Cancer Society and the Nordic Cancer Union. HERPACC was supported by a Grant-in-Aid for Scientific Research on Priority Areas and on Innovative Area from the Ministry of Education, Science, Sports, Culture and Technology of Japan and by a Grant-in-Aid for the Third Term Comprehensive 10-Year Strategy for Cancer Control from Ministry Health, Labour and Welfare of Japan. HMBCS was supported by short-term fellowships from the German Academic Exchange Program (NWB) and the Friends of Hannover Medical School (NWB). KBCP was financially supported by the special Government Funding (EVO) of Kuopio University Hospital grants, Cancer Fund of North Savo, the Finnish Cancer Organizations, the Academy of Finland and by the strategic funding of the University of Eastern Finland. kConFab is supported by funding from the National Breast Cancer Foundation, the NHMRC, the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia and the Cancer Foundation of Western Australia. The kConFab Clinical Follow Up Study was funded by the NHMRC (145684, 288704, 454508). Financial support for the AOCs was provided by the United States Army Medical Research and Materiel Command (DAMD17-01-1-0729), the Cancer Council of Tasmania and Cancer Foundation of Western Australia and the NHMRC (199600). GC-T and P Webb are supported by the NHMRC. LAABC is supported by grants (18B-0287, 3PB-0102, 5PB-0018, 10PB-0098) from the California Breast Cancer Research Program. Incident breast cancer cases were collected by the USC Cancer Surveillance Program (CSP), which is supported under subcontract by the California Department of Health. CSP is also part of the National Cancer Institute’s Division of Cancer Prevention and Control Surveillance, Epidemiology, and End Results Program, under contract number N01CN25403. LMC is supported by the ‘Stichting tegen Kanker’ (232-2008 and 196-2010). DL is supported by the KULPEV/10/016-SymBioSys.

The MARE study was supported by the Deutsche Krebsforsch e v. (70-2892-BI), the Hamburg Cancer Society, the German Cancer Research Center and the genotypic work in part by the Federal Ministry of Education and Research (BMBF) Germany (01K1H0402). GCBS was funded by grants from Italian Association for Cancer Research (AIRC, IG B213) and by Italian citizens who allocated the $1,000 share of their tax payment in support of the Fondazione IRCCS Istituto Nazionale dei Tumori, according to Italian laws (INT-Institutional strategic projects 5x1000). MCBS was supported by the NIH grants CA116167 and CA128978, an NIH Specialized Program of Research Excellence (SPORE) in Breast Cancer (CA116201), the Breast Cancer Research Foundation, and a generous gift from the David F and Margaret S. Grunfeld Family Foundation and the Ling-Tsung and Wei Feng Cancer Chair of West China Medical School. GCBS was supported by the United States National Cancer Institute grants (grants R01CA64277, R01CA148667, and R37CA70867). Biological sample preparation was conducted the Survey and Biospecimen Shared Resource, which is supported by P30 CA68485. SBCS was supported by Yorkshire Cancer Research S295, S299, S305PA. SBCGS is supported by a grant from the
University of Southern California, 1975 Zonal Ave, Los Angeles, CA 90033, USA. 64Laboratory for Translational Genetics, Department of Oncology, University of Leuven, Oude Markt 13  bus 5005, 3000 Leuven, Belgium. 65Vesalius Research Center, VIB, Herestraat 49, box 912, Onderwijs & Navoring 4, Building 404-24, 3000 Leuven, Belgium. 66Multidisciplinary Breast Center, University Hospital Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium. 67Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. 68Department of Cancer Epidemiology/Clinical Cancer Registry, University Clinic Hamburg-Eppendorf, Martinistrasse 52, D - 20246 Hamburg, Germany. 69Institute for Medical Biometrics and Epidemiology, University Clinic Hamburg-Eppendorf, Martinistrasse 52, D - 20246 Hamburg, Germany. 70Unit of Molecular Bases of Genetic Risk and Genetic Testing, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Via Venezian 1, 20133 Milan, Italy. 71FOM, Fondazione Istituto FIRC di Oncologia Molecolare, Via Adarnello 16, 20139 Milan, Italy. 72Division of Cancer Prevention and Genetics, Istituto Europeo di Oncologia (IEO), Via Giuseppe Ripamonti 435, 20141 Milan, Italy. 73Cogentech Cancer Genetic Test Laboratory, 111 Luton Campi, University of York, University of York. 74Department of Laboratory Medicine and Pathology, Division of Experimental Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. 75Department of Health Sciences Research, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. 76Cancer Epidemiology Centre, The Cancer Council Victoria, 615 St Kilda Road, Melbourne, Victoria 3004, Australia. 77Department of Medicine, McGill University Health Centre, Montreal, Quebec. 78McGill University Health Centre, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec H3A 1A1, Canada. 79Department of Social and Preventive Medicine and Department of Environmental and Occupational Health at Work, University of Montreal, Marguerite d'Youville Pavilion, 2375 Côte Ste-Catherine, Suite 4095, Montréal, Québec H3T 1A8, Canada. 80Cancer Genomics Laboratory, Centre Hospitalier Universitaire de Québec, Research Center and Laval University, 2325 Rue de l'Université, Québec City, Quebec, G1V 0A6, Canada. 81Breast Cancer Research Unit, Department of Cancer Research, University of Malaya Cancer Research Institute, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia. 82Cancer Research Initiatives Foundation, Sime Darby Medical Centre Subang Jaya, 1, Jalan SS 12 / 1A, 47500 Subang Jaya, Selangor Darul Ehsan, Malaysia. 83Singapore Cancer Research Institute, National University of Singapore, Singapore National Eye Centre, 11 Third Hospital Avenue, Singapore 168751, Singapore. 84Department of Genetics, Institute for Cancer Research, Oslo University Hospital, The Norwegian Radium Hospital, N-0316 Oslo, Norway. 85Faculty of Medicine (Faculty Division Ahus), University of Oslo, Sogn Arena, Klaus Torgård's vei 3, 2. etg, 0372 Oslo, Norway. 86Division of Epidemiology, Department of Medicine, Vanderbilt Biomedical Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, 1161 21st Ave S # T1217, Nashville, TN 37232, USA. 87Laboratory of Cancer Genetics and Tumor Biology, Department of Clinical Chemistry and Biocenter Oulu, University of Oulu, Oulu University Hospital, Kajaaniinte 50, 90220 Oulu, Finland. 88Department of Oncology, Oulu University Hospital, University of Oulu, Kajaaniinte 50, 90220 Oulu, Finland. 89Department of Surgery, Oulu University Hospital, University of Oulu, Kajaaniinte 50, 90220 Oulu, Finland. 90Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 982 - 600 University Avenue, Toronto, Ontario M5G 1X5, Canada. 91Department of Molecular Genetics, University of Toronto, Medical Science Building, Room 4386, 1 King's College Cir, Toronto, Ontario M5S 1A8, Canada. 92Division of Epidemiology, School of Public Health, University of Illinois, 601 South Wood Street, 6th floor, 155 College St, Toronto, Ontario MST 3M7, Canada. 93Ontario Cancer Genetics Network, 620 University Avenue, Toronto, Ontario MST 2L7, Canada. 94Department of Laboratory Medicine and Pathobiology, University of Toronto, Medical Sciences Building, 6th Floor, 1 King's College Cir, Toronto, Ontario M5S 1A8, Canada. 95University Health Network, R. Fraser Elliott Building, 1st Floor, 190 Elizabeth St., Toronto, Ontario M5G 2C4, Canada. 96Department of Human Genetics & Department of Pathology, Leiden University Medical Centre, Eindhovenseweg 20, 2333 ZC, Leiden, The Netherlands. 97Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, Rockville, MD 20850, USA. 98Department of Cancer Epidemiology and Prevention, M. Sklodowska-Curie Memorial Cancer Centre & Institute of Oncology, Roentgena 5, 02-781 Warsaw, Poland. 99Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Solnavägen 1, Stockholm 17177, Sweden. 100Department of Medical Oncology, Family Cancer Clinic, Erasmus University Medical Center, Groene Hilledijk 301, 3075EA, Rotterdam, The Netherlands. 101Department of Medical Oncology, Josephine Nefkens Institute, Erasmus University Medical Center, Groene Hilledijk 301, 3075 EA, Rotterdam, The Netherlands. 102Department of Clinical Genetics, Family Cancer Clinic, Erasmus University Medical Center, Groene Hilledijk 301, 3075 EA, Rotterdam, The Netherlands. 103Human Genetics Division, Genome Institute of Singapore, 60 Biopolis St, Singapore 138672, Singapore. 104Institute for Cancer Studies, Department of Oncology, CRUK/YCR Shefﬁeld Cancer Research Centre, University of Shefﬁeld, 385a Glossop Road, Shefﬁeld S10 2HQ, UK. 105Academic Unit of Surgical Oncology, Department of Oncology, CRUK/YCR Shefﬁeld Cancer Research Centre, University of Shefﬁeld, 385a Glossop Road, Shefﬁeld S10 2HQ, UK. 106Academic Unit of Pathology, Department of Neurosurgery, University of Shefﬁeld, 385a Glossop Road, Shefﬁeld S10 2HQ, UK. 107International Epidemiology Institute, 1455 Research Blvd, Rockville, MD 20850, USA. 108Department of Epidemiology, Harvard School of Public Health, 67 Huntington Avenue, Boston, MA 02115, USA. 109Channing Division of Network Medicine, Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115, USA. 110Dana-Farber/Harvard Cancer Center, 450 Brookline Ave, Boston, MA 02215, USA. 111Department of Surgery, Tri-Services General Hospital, National Defense Medical Center, Taipei, Taiwan. 112Department of Surgery, National Taiwan University Hospital, No.1, Chung-Shan S Road, Taipei City 101, Taiwan. 113International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon, CEDEX 08, France. 114Division of General Surgery, Yong Loo Lin School of Medicine, National University of Singapore, 1E, Kent Ridge Road, Singapore 119228, Singapore. 115National University Health System, 1E, Kent Ridge Road, Singapore 119228, Singapore. 116Saw Swee Hock School of Public Health, National University of Singapore, MD3, 16 Medical Drive, Singapore 117597, Singapore. 117Division of General Surgery, National University Health System, 1E, Kent Ridge Road, Singapore 119228, Singapore. 118Molecular Genetics of Breast Cancer, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. 119Center for Primary Health Care Research, University of Lund, Paradisgatan 5, SE-221 00 Lund, Malmö, Sweden. 120Institute of Pathology, Städtisches Klinikum Karlsruhe, Motzkestrasse 90, 76133 Karlsruhe, Germany. 121Frauenklinik der Städtklinik Baden-Baden, Balger Strasse 50, 76532 Baden-Württemberg, Germany. 122Department of Genetics and Pathology, Pomeranian Medical University, Rybacka 1, 70-204 Szczecin, Poland. 123Postgraduate School of Molecular Medicine, Warsaw Medical University, Zwirki i Wschow 61, 02-091 Warsaw, Poland. 124National Cancer Institute, 268/ 1 Rama VI Road, Rajathevi, Bangkok 10400, Thailand. 125International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon, CEDEX 08, France. 126Department of Molecular Virology, Immunology and Medical Genetics, Comprehensive Cancer Center, The Ohio State University, 410 W. 10th Avenue, Columbus, OH 43210, USA. 127Molecular Diagnostics Laboratory, IRPP, National Centre for Scientific Research “Demokritos”, Aghia Paraskevi Attikis 153 10, Athens, Greece. 128College of Public Health, China Medical University, No.91, Hsin-Hsin Road, Taichung 40402, Taiwan. 129Institute of Biomedical Sciences, Academia Sinica, 2 Academia Road, Nangang, Taipei 115, Taiwan. 130Department of Surgery, Tri-Service General Hospital, No.325, Sec.2 Chenggong RoadNeihu District, Taipei City 114, Taiwan. 131Department of Surgery, National Taiwan University Hospital, No.1, Changde StreetZhongzheng District, Taipei City 10048, Taiwan. 132Cancer Center, Kaohsiung Medical University Chung-Ho Memorial Hospital, No.100, Tzuoy 1st Road, Kaohsiung 807, Taiwan. 133Department of Surgery, Kaohsiung Medical University Chung-Ho Memorial Hospital, No.100, Tzuoy 1st Road, Kaohsiung 807, Taiwan. 134McGill University and Genome Québec Innovation Centre, 740, Dr. Penfield Avenue, Room 7104, Montréal, Québec H3A 0G1, Canada. 135Department of Public Health and Primary Care, Centre for Cancer Genetic Epidemiology, University of Cambridge, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 8RN, UK.
References


Cite this article as: Johnson et al.: Genetic variation at CYP3A4 is associated with age at menarche and breast cancer risk: a case-control study. Breast Cancer Research 2014 16:R51.

doi:10.1186/bcr3662

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit