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Polymorphisms of the nuclear receptor pregnane X receptor and organic anion transporter polypeptides 1A2, 1B1, 1B3 and 2B1 are not associated with breast cancer risk

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Key words: PXR; OATP; SLCO; polymorphisms; breast cancer risk

Abstract

Organic anion transporter polypeptides (OATPs, *SLCOs*) are involved in the uptake of conjugates steroid hormones such as estrone-3-sulfate. It has been suggested that the expression of OATPs in breast tissues could impact breast carcinogenesis and tumor pathology. The nuclear receptor pregnane X receptor PXR is involved in the regulation of *SLCO1A2* expression. We investigated 31 variants located in *PXR*, *SLCO1A2*, *SLCO1B1*, *SLCO1B3* and *SLCO2B1* for an association with breast cancer risk and/or histo-pathological tumor characteristics. Polymorphisms were selected on the basis of a known or potential functional consequence and an allele frequency >2%. Genotyping was performed by matrix assisted laser desorption/ionization time-of-flight mass spectrometry using the GENICA population-based breast cancer case control collection comprising 1021 cases and 1015 age matched controls. Statistical analysis was performed by SAS and all tests were two-sided. None of the 31 analyzed transporter and PXR polymorphisms showed an association with breast cancer risk or tumor characteristics. Our data suggest that among the many known transporters common variations of *PXR*, *SLCO1A2*, *SLCO1B1*, *SLCO1B3* and *SLCO2B1* do not contribute to breast carcinogenesis.

Introduction

The mammary gland is an estrogen target tissue and plasma levels of estradiol have been linked with breast cancer risk [1]. The metabolite estrone-3-sulfate is considered one of the major precursors of active estrogen in postmenopausal women [2]. Estrone-3-sulfate levels are significantly higher in malignant tissue, resulting in increased levels of 17 β -estradiol [3]. Due to the hydrophilic nature of this compound it is likely that an uptake transporter is needed to facilitate the transmembrane entry into tissue such as breast [4]. Recently, the interplay between the nuclear receptor pregnane X receptor (PXR) and the uptake transporter organic anion transporter polypeptide 1A2 (OATP1A2, gene symbol *SCLO1A2*) has been reported to enhance estrogen effects in breast cancer [5]. The induction of OATP1A2 by the PXR activator rifampin was associated with increased uptake of estrone-3-sulfate, an effect that could be abrogated by small interfering RNA targeting the PXR. The identification of a PXR response element in the *SCLO1A2* gene followed by PXR-*SCLO1A2* chromatin immunoprecipitation with anti PXR and the reversal of the effect by a potent PXR antagonist substantiated the cooperation between PXR and OATP1A2 [5]. mRNA and protein expression in breast cancer revealed closely correlated high PXR and OATP1A2 levels and suggested that PXR and its target gene OATP1A2 are histo-pathological markers of dedifferentiation and progression [6].

The organic anion uptake transporter polypeptides (OATPs encoded by the *SLCOs* genes) belong to the superfamily of solute carriers [7-9]. They are expressed in various tissues including liver, kidney, brain, testis as well as breast and mediate the cellular uptake of a broad range of endogenous substrates (e.g. bile acids, thyroid hormones, conjugated steroids including estradiol-17 β -glucuronide and estrone-3-sulfate) and drugs (e.g. antibiotics, nonsteroidal anti-inflammatory drugs, anti-cancer drug methotrexate) [10]. A number of naturally occurring sequence variants has been identified in OATPs [11, 12] and PXR [13, 14] several of which are relatively common. In the case of OATP1B1, a hepatic estrone-3-sulfate

uptake transporter, the genetic variations (e.g. 521 T>C, rs4149056) have been linked with decreased transport activity for an irinotecan derivative SN-38, pravastatin, estrone-3-sulfate and estradiol-17beta-glucuronide [15-17]. Since this OATP variant may alter the pharmacokinetics of drugs thereby contributing to the inter-individual variability of drug response and/or the development of side effects (e.g. statin-related myopathy), it is plausible to also question a putative role of uptake transporters for breast cancer risk [16, 18, 19]. Of note, estrone-3-sulfate is a substrate for a number of OTAPs including OATP1A2, OATP1B1, OATP1B3 as well as OATP2B1 and for all of these transporters functional relevant genetic variants have been reported [10, 20-23]. This notion is supported by recent findings from a whole genome scan and association study conducted with more than 37,000 cases and 40,000 controls that revealed the sodium bicarbonate co-transporter *SLC4A7* variant rs4973768 to be associated with increased breast cancer risk and tumor estrogen receptor status [24]. Given the biological plausibility and examples of transporter variants involved in health and disease we investigated whether variants at *SLCOs* as well as the xenobiotic and steroid hormone sensing *PXR* may impact breast cancer risk and/or histo-pathological tumor characteristics. We explored this possibility for 23 *SCLO* polymorphisms (six at *SLCO1A2*, six at *SLCO1B1*, three at *SLCO1B3*, seven at *SLCO2B1*) and eight *PXR* polymorphisms for which functional consequences are known or suggested. Association studies have been performed with the population-based GENICA breast cancer case-control collection (1021 cases and 1015 controls) from Germany. None of the polymorphisms showed an association neither with breast cancer risk nor with histo-pathological tumor characteristics.

Material and methods

Study population

The GENICA study participants of the population-based breast cancer case-control study from the Greater Bonn Region, Germany, were recruited between 08/2000 and 9/2004 as described previously [25, 26]. In brief, there are 1143 incident breast cancer cases and 1155 population controls matched in 5-year classes. Cases and controls were eligible if they were of Caucasian ethnicity, current residents of the study region and below 80 years of age. Information on known and supposed risk factors was collected for all participants via in-person interviews. The response rate for cases was 88% and for controls 67%. DNA samples were available for 1021 cases (89%) and 1015 controls (88%). Characteristics of the study population regarding potential breast cancer risk factors include age at diagnosis (<50, ≥50 years), menopausal status (premenopausal, postmenopausal), family history of at least one first degree relative with breast or ovarian cancer (yes, no), use of oral contraceptives (never, >0-<5, 5-<10, ≥10 years), use of hormone therapy (never, >0-<10, ≥10 years), body mass index (<20, 20-<25, 25-<30, ≥30 kg/m²) and smoking status (never, former, current) (Table 1).

Information on clinical and histo-pathological tumor characteristics was available for 1011 (99%) breast cancer cases. The dataset included estrogen receptor status (positive, negative), progesterone receptor status (positive, negative), grading (G1, G2, G3), tumor size (T1, T2, T3, T4), histology (ductal, lobular, ductolobular) and nodal status (N0, N≥1) (Table 1).

The GENICA study was approved by the Ethic's Committee of the University of Bonn. All study participants gave written informed consent.

Isolation of DNA and genotyping

Genomic DNA was extracted from heparinized blood samples (PuregeneTM, Gentra Systems, Inc., Minneapolis, USA) as previously described [27]. Twenty-three polymorphisms located in *SLCO1A2*, *SLCO1B1*, *SLCO1B3*, and *SLCO2B1* and eight polymorphisms in *PXR* were selected on the basis of a known or a potential functional consequence and an allele frequency of at least 2% in Caucasians. The polymorphisms *SLCO1A2*_-6159_A>G (rs11830638), *SLCO1A2*_-5948_G>A (rs4762818), *SLCO1A2*_-2800_A>G (rs2306231), *SLCO1A2*_-423_G>A (rs3764043), *SLCO1A2*_37_A>G (rs10841795), *SLCO1A2*_516_T>G (rs11568563), *SLCO1B1*_-11939_T>C (rs17328763), *SLCO1B1*_-2229_C>T (rs3829306), *SLCO1B1*_388_G>A (rs2306283), *SLCO1B1*_463_C>A (rs11045819), *SLCO1B1*_521_T>C (rs4149056), *SLCO1B1*_1929_C>A (rs34671512), *SLCO1B3*_-3954_G>A (rs7962265), *SLCO1B3*_-1390_G>A (rs2138334), *SLCO1B3*_334_G>T (rs4149117), *SLCO2B1*_-8614_A>G (rs2851104), *SLCO2B1*_-5910_T>G (rs11236349), *SLCO2B1*_-4142_A>T (rs2712805), *SLCO2B1*_-2366_G>T (rs2851064), *SLCO2B1*_-1011_A>C (rs4100076), *SLCO2B1*_-71_C>T (rs2851069), *SLCO2B1*_935_G>A (rs12422149), *SLCO2B1*_1457_C>T (rs2306168), *PXR*_-25385_C>T (rs3814055), *PXR*_-24381_A>C (rs1523127), *PXR*_-24113_G>A (rs2276706), *PXR*_252_A>G (rs1464603), *PXR*_7635_A>G (rs6785049), *PXR*_8055_C>T (rs2276707), *PXR*_10799_G>A (rs1054191) and *PXR*_11156_A>C (rs3814057) were subjected to genotyping of all 2036 DNA samples which are available from the GENICA collection. Genotyping was performed by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) as described previously [27, 28]. A Sequenom Compact MALDI-TOF MS was used for data acquisitions from the SpectroCHIP. Genotyping calls were made with MASSARRAY RT software v 3.0.0.4 (Sequenom, San Diego, CA, USA). For quality control repeated analyses were performed for 20% randomly selected samples. Primers were synthesized by Metabion International AG, Martinsried, Germany, sequences are available on request.

Statistical analyses

SLCO1A2, *SLCO1B1*, *SLCO1B3*, *SLCO2B1* and *PXR* genotype frequencies were tested for Hardy-Weinberg equilibrium. Associations between genetic variables and breast cancer risk were analyzed by conditional logistic regression adjusted for six potential epidemiological breast cancer risk factors (menopausal status, family history of breast cancer, use of oral contraceptives, use of hormone therapy, body mass index and smoking). Subsequently subgroup analysis was performed for these six epidemiological variables. Additionally, the associations between *SLCO1A2*, *SLCO1B1*, *SLCO1B3*, *SLCO2B1* and *PXR* genotypes and seven clinical and histo-pathological tumor characteristics (estrogen receptor status, progesterone receptor status, HER2 status, grading, tumor size, histology and node status) of breast cancer cases were analyzed by conditional logistic regression and chi square test. All tests were two-sided. To correct for multiple testing we divided the significance level of 0.05 by the number of tested variables. In case of epidemiological variables 0.05 was divided by six and thus P -values < 0.008 were considered significant. Accordingly for the seven tumor characteristics P -values < 0.007 were considered significant. Risk estimates were given as odds ratios (OR) and 95% confidence interval (CI). Haplotypes of *SLCO1A2*, *SLCO1B1*, *SLCO1B3*, *SLCO2B1* and *PXR* were estimated using Haploview 4.1 [29]. All other statistical analyses were done using SAS v 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results

We analyzed six polymorphisms located in *SLCO1A2*, six polymorphisms located in *SLCO1B1*, three polymorphisms located in *SLCO1B3*, eight polymorphisms located in

SLCO2B1 and eight polymorphisms located in *PXR* using MALDI-TOF MS. Call rates were >98% and repeated analyses of 20% of randomly selected samples showed 100% concordance. Genotype frequencies of cases and controls met Hardy-Weinberg equilibrium. Statistical analyses revealed that none of these polymorphisms is association with breast cancer risk in general (Table 2) or in subgroup analyses with respect to menopausal status, family history of breast cancer, use of oral contraceptives, use of hormone therapy, body mass index and smoking (data not shown). Moreover, none of the 31 polymorphisms showed an association with histo-pathological characteristics of breast tumors considering estrogen receptor, progesterone receptor and HER2 status, grading, tumor size, histology as well as node status (data not shown). Furthermore, haplotype analyses revealed no association between *SLCO1A2*, *SLCO1B1*, *SLCO1B3*, *SLCO2B1* and *PXR* haplotypes and breast cancer risks (data not shown).

Discussion

To the best of our knowledge this is the first study on a potential role of *SLCO* and *PXR* polymorphisms in breast cancer risk and/or histo-pathological tumor characteristics. We challenged this question due to known biological roles of OATP2 and PXR in breast cancer as well as known pharmacokinetic effects of OATP1B1 variants [5, 6, 15-17]. Yet, none of the investigated polymorphisms in *SLCO1A2*, *SLCO1B1*, *SLCO1B3* and *SLCO2B1* and none of the investigated *PXR* polymorphisms was associated with over all breast cancer risk or any of the subgroup risks addressed by menopausal status, family history of breast cancer, use of oral contraceptives, use of hormone therapy, body mass index and smoking. With respect to breast tumor characteristics i.e. estrogen receptor, progesterone receptor and HER2 status, grading, tumor size, histology and nodal status also no association has been observed. Our study had an 80 % power to detect a minimum OR of 1.4 for the 31 polymorphisms ($\alpha = 0.05$, two sided

test). Our finding of a lack of breast cancer risk association with *SLCO1A2*, *SLCO1B1*, *SLCO1B3* and *SLCO2B1* as well as *PXR* polymorphisms is of relevance because transporters and their translational modifiers are key players in the uptake and elimination of endogenous substrates, xenobiotics and drugs. While we limited our analyses to polymorphism of a small number of uptake transporters of organic anions (OATPs) it is of note that at least 5% (>2000) of all genes are transporter related consistent with their biological significance and their role in cell homeostasis [9]. We therefore cannot exclude that breast cancer susceptibility and/or tumor characteristics may be conferred by variants in other solute carriers (e.g. other OATPs, OATs, OCTs) and/or in ATP-binding cassette membrane transporters (e.g. ABCB and ABCC) or variants in other related gene [10, 20], as is has been shown for sodium bicarbonate co-transporter *SLC4A7* [24].

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All authors disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations.

References

1. Key T, Appleby P, Barnes I, Reeves G (2002) Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst* 94:606-16.
2. Zhu BT, Conney AH (1998) Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis* 19:1-27.
3. Nozawa T, Suzuki M, Yabuuchi H, Irokawa M, Tsuji A, Tamai I (2005) Suppression of cell proliferation by inhibition of estrone-3-sulfate transporter in estrogen-dependent breast cancer cells. *Pharm Res* 22:1634-41.
4. Nozawa T, Suzuki M, Takahashi K, Yabuuchi H, Maeda T, Tsuji A, Tamai I (2004) Involvement of estrone-3-sulfate transporters in proliferation of hormone-dependent breast cancer cells. *J Pharmacol Exp Ther* 311:1032-7.
5. Meyer zu Schwabedissen HE, Tirona RG, Yip CS, Ho RH, Kim RB (2008) Interplay between the nuclear receptor pregnane X receptor and the uptake transporter organic anion transporter polypeptide 1A2 selectively enhances estrogen effects in breast cancer. *Cancer Res* 68:9338-47.
6. Miki Y, Suzuki T, Kitada K, Yabuki N, Shibuya R, Moriya T, Ishida T, Ohuchi N, Blumberg B, Sasano H (2006) Expression of the steroid and xenobiotic receptor and

its possible target gene, organic anion transporting polypeptide-A, in human breast carcinoma. *Cancer Res* 66:535-42.

7. Hagenbuch B, Meier PJ (2004) Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. *Pflugers Arch* 447:653-65.
8. Hagenbuch B, Gui C (2008) Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. *Xenobiotica* 38:778-801.
9. Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, Bruford EA (2004) The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteinsIntroduction. *Pflugers Arch* 447:465-8.
10. Nies AT, Schwab M, Keppler D (2008) Interplay of conjugating enzymes with OATP uptake transporters and ABCC/MRP efflux pumps in the elimination of drugs. *Expert Opin Drug Metab Toxicol* 4:545-68.
11. Iida A, Saito S, Sekine A, Mishima C, Kondo K, Kitamura Y, Harigae S, Osawa S, Nakamura Y (2001) Catalog of 258 single-nucleotide polymorphisms (SNPs) in genes encoding three organic anion transporters, three organic anion-transporting polypeptides, and three NADH:ubiquinone oxidoreductase flavoproteins. *J Hum Genet* 46:668-83.
12. Seithel A, Glaeser H, Fromm MF, König J (2008) The functional consequences of genetic variations in transporter genes encoding human organic anion-transporting polypeptide family members. *Expert Opin Drug Metab Toxicol* 4:51-64.
13. Hustert E, Zibat A, Presecan-Siedel E, Eiselt R, Mueller R, Fuss C, Brehm I, Brinkmann U, Eichelbaum M, Wojnowski L, Burk O (2001) Natural protein variants

of pregnane X receptor with altered transactivation activity toward CYP3A4. *Drug Metab Dispos* 29:1454-9.

14. Zhang J, Kuehl P, Green ED, Touchman JW, Watkins PB, Daly A, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Wrighton SA, Hancock M, Kim RB, Strom S, Thummel K, Russell CG, Hudson JR, Jr., Schuetz EG, Boguski MS (2001) The human pregnane X receptor: genomic structure and identification and functional characterization of natural allelic variants. *Pharmacogenetics* 11:555-72.
15. Iwai M, Suzuki H, Ieiri I, Otsubo K, Sugiyama Y (2004) Functional analysis of single nucleotide polymorphisms of hepatic organic anion transporter OATP1B1 (OATP-C). *Pharmacogenetics* 14:749-57.
16. Nozawa T, Minami H, Sugiura S, Tsuji A, Tamai I (2005) Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: in vitro evidence and effect of single nucleotide polymorphisms. *Drug Metab Dispos* 33:434-9.
17. Tirona RG, Leake BF, Merino G, Kim RB (2001) Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J Biol Chem* 276:35669-75.
18. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M, Collins R (2008) SLCO1B1 variants and statin-induced myopathy--a genomewide study. *N Engl J Med* 359:789-99.
19. Niemi M (2007) Role of OATP transporters in the disposition of drugs. *Pharmacogenomics* 8:787-802.
20. *Drug Transporters*, Edited by You G and Morris ME(2007) WILEY.

21. Franke RM, Scherkenbach LA, Sparreboom A (2009) Pharmacogenetics of the organic anion transporting polypeptide 1A2. *Pharmacogenomics* 10:339-44.
22. Kalliokoski A, Niemi M (2009) Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* 158:693-705.
23. Meyer zu Schwabedissen HE, Kim RB (2009) Hepatic OATP1B transporters and nuclear receptors PXR and CAR: interplay, regulation of drug disposition genes, and single nucleotide polymorphisms. *Mol Pharm* 6:1644-61.
24. Ahmed S, Thomas G, Ghousaini M, Healey CS, Humphreys MK, Platte R, Morrison J, Maranian M, Pooley KA, Luben R, Eccles D, Evans DG, Fletcher O, Johnson N, Dos SS, I, Peto J, Stratton MR, Rahman N, Jacobs K, Prentice R, Anderson GL, Rajkovic A, Curb JD, Ziegler RG, Berg CD, Buys SS, McCarty CA, Feigelson HS, Calle EE, Thun MJ, Diver WR, Bojesen S, Nordestgaard BG, Flyger H, Dork T, Schurmann P, Hillemanns P, Karstens JH, Bogdanova NV, Antonenkova NN, Zalutsky IV, Bermisheva M, Fedorova S, Khusnutdinova E, Kang D, Yoo KY, Noh DY, Ahn SH, Devilee P, van Asperen CJ, Tollenaar RA, Seynaeve C, Garcia-Closas M, Lissowska J, Brinton L, Peplonska B, Nevanlinna H, Heikkinen T, Aittomaki K, Blomqvist C, Hopper JL, Southey MC, Smith L, Spurdle AB, Schmidt MK, Broeks A, van Hien RR, Cornelissen S, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Schmutzler RK, Burwinkel B, Bartram CR, Meindl A, Brauch H, Justenhoven C, Hamann U, Chang-Claude J, Hein R, Wang-Gohrke S, Lindblom A, Margolin S, Mannermaa A, Kosma VM, Kataja V, Olson JE, Wang X, Fredericksen Z, Giles GG, Severi G, Baglietto L, English DR, Hankinson SE, Cox DG, Kraft P, Vatten LJ, Hveem K, Kumle M, Sigurdson A, Doody M, Bhatti P, Alexander BH, Hooning MJ, van den Ouweland AM, Oldenburg RA, Schutte M, Hall P, Czene K, Liu J, Li Y, Cox A, Elliott G, Brock I, Reed MW, Shen CY, Yu JC, Hsu GC, Chen ST, nton-Culver H,

- Ziogas A, Andrulis IL, Knight JA, Beesley J, Goode EL, Couch F, Chenevix-Trench G, Hoover RN, Ponder BA, Hunter DJ, Pharoah PD, Dunning AM, Chanock SJ, Easton DF (2009) Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat Genet* 41:585-90.
25. Justenhoven C, Pierl CB, Haas S, Fischer HP, Baisch C, Hamann U, Harth V, Pesch B, Bruning T, Vollmert C, Illig T, Dippon J, Ko YD, Brauch H (2008) The CYP1B1_1358_GG genotype is associated with estrogen receptor-negative breast cancer. *Breast Cancer Res Treat* 111:171-7.
26. Pesch B, Ko Y, Brauch H, Hamann U, Harth V, Rabstein S, Pierl C, Fischer HP, Baisch C, Justenhoven C, Ranft U, Bruning T (2005) Factors modifying the association between hormone-replacement therapy and breast cancer risk. *Eur J Epidemiol* 20:699-711.
27. Justenhoven C, Hamann U, Pesch B, Harth V, Rabstein S, Baisch C, Vollmert C, Illig T, Ko YD, Bruning T, Brauch H (2004) ERCC2 genotypes and a corresponding haplotype are linked with breast cancer risk in a German population. *Cancer Epidemiol Biomarkers Prev* 13:2059-64.
28. Schaeffeler E, Zanger UM, Eichelbaum M, sante-Poku S, Shin JG, Schwab M (2008) Highly multiplexed genotyping of thiopurine s-methyltransferase variants using MALDI-TOF mass spectrometry: reliable genotyping in different ethnic groups. *Clin Chem* 54:1637-47.
29. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263-5.

Table 1 Breast tumor characteristics and epidemiologic baseline information of the GENICA study population

		Cases		Controls		OR ^a (95% CI)
		n	(%)	n	(%)	
Tumor characteristics						
Estrogen receptor status	positive	755	(77.8)			
	negative	216	(22.2)			
Progesterone receptor status	positive	678	(70.0)			
	negative	291	(30.0)			
Grading	G1	77	(8.2)			
	G2	567	(60.4)			
	G3	295	(31.4)			
Tumor size	T1	582	(61.9)			
	T2	289	(30.7)			
	T3	30	(3.2)			
	T4	39	(4.2)			
Histology	ductal	634	(69.5)			
	lobular	177	(19.4)			
	ductolobular	101	(11.1)			
Nodal status	N0	602	(63.8)			
	≥ N1	342	(36.2)			
Epidemiological variables						
Age (years)	< 50	225	(22.6)	226	(22.3)	
	≥ 50	796	(77.3)	789	(77.7)	
Menopausal status	Pre	248	(24.8)	235	(23.6)	1.00 ^b
	Post	753	(75.2)	762	(76.4)	0.90 (0.65-1.24)
Family history of breast cancer	No	845	(84.4)	914	(91.7)	1.00 ^b
	Yes	156	(15.6)	83	(8.3)	2.04 (1.53-2.70)
Use of oral contraceptives (years)	Never	372	(36.5)	368	(36.3)	1.00 ^b
	> 0 < 5	180	(17.7)	185	(18.3)	0.97 (0.74-1.28)
	5 < 10	134	(13.1)	120	(11.8)	1.11 (0.81-1.52)
	≥ 10	333	(32.7)	340	(33.6)	0.97 (0.76-1.25)
Use of hormone replacement therapy (years)	Never	506	(49.8)	509	(50.3)	1.00 ^b
	> 0 < 10	245	(24.1)	290	(28.6)	0.86 (0.68-1.09)
	≥ 10	266	(26.1)	214	(21.1)	1.36 (1.05-1.76)
Body mass index (kg/m ²)	< 20	88	(8.8)	70	(7.2)	1.28 (0.91-1.81)
	20 < 25	459	(45.9)	464	(46.4)	1.00 ^b
	25 < 30	302	(30.1)	319	(32.0)	0.99 (0.80-1.22)
	≥ 30	152	(15.2)	144	(14.4)	1.08 (0.83-1.42)
Smoking	Never	586	(57.5)	555	(54.7)	1.00 ^b
	Former	192	(18.8)	215	(21.2)	0.95 (0.75-1.19)
	Current	242	(23.7)	245	(24.1)	0.84 (0.66-1.06)

The table includes all patients for whom genomic DNA was available.

Abbreviations: CI = confidence interval, OR = odds ratio

^aOR conditional on age in 5-year groups adjusted for menopausal status, family history of breast cancer, use of oral contraceptives, use of hormone replacement therapy, body mass index, and smoking

^bReference

Table 2. Genotype frequencies and risk estimates of polymorphisms located in *SLCO1A2*, *SLCO1B1*, *SLCO1B3*, *SLCO2B1* and *PXR* in breast cancer cases and controls

	Genotype	Controls n (%)	Cases n (%)	OR (95% CI) ^a
<i>SLCO1A2</i> _-6159_A>G (rs11830638)	AA	770 (77.1)	781 (77.0)	1.00 ^b
	AG	209 (21.0)	220 (21.7)	1.07 (0.86-1.33)
	GG	19 (1.9)	13 (1.3)	0.69 (0.33-1.45)
<i>SLCO1A2</i> _-5948_G>A (rs4762818)	GG	771 (38.3)	781 (38.8)	1.00 ^b
	GA	211 (21.1)	223 (22.0)	1.08 (0.87-1.34)
	AA	17 (1.7)	11 (1.1)	0.65 (0.29-1.46)
<i>SLCO1A2</i> _-2800_A>G (rs2306231)	AA	478 (47.9)	490 (48.9)	1.00 ^b
	AG	417 (41.7)	415 (41.4)	0.97 (0.80-1.17)
	GG	104 (10.4)	97 (9.7)	0.89 (0.65-1.21)
<i>SLCO1A2</i> _-423_G>A (rs3764043)	GG	962 (96.8)	971 (96.2)	1.00 ^b
	GA	32 (3.2)	37 (3.7)	1.13 (0.69-1.85)
	AA	0	1 (0.1)	-
<i>SLCO1A2</i> _37_A>G (rs10841795)	AA	752 (75.3)	751 (74.0)	1.00 ^b
	AG	228 (22.9)	239 (23.6)	1.02 (0.82-1.26)
	GG	18 (1.8)	24 (2.4)	1.35 (0.72-2.51)
<i>SLCO1A2</i> _516_T>G (rs11568563)	TT	897 (89.4)	889 (88.5)	1.00 ^b
	TG	106 (10.6)	116 (11.5)	1.13 (0.85-1.50)
	GG	0	0	-
<i>SLCO1B1</i> _-11939_T>C (rs17328763)	TT	668 (67.8)	649 (66.4)	1.00 ^b
	TC	290 (29.4)	301 (30.8)	1.06 (0.87-1.29)
	CC	28 (2.8)	43 (2.8)	1.46 (0.89-2.40)
<i>SLCO1B1</i> _-2229_C>T (rs3829306)	CC	872 (88.2)	870 (87.4)	1.00 ^b
	CT	112 (11.3)	124 (12.5)	1.14 (0.86-1.49)
	TT	5 (0.5)	1 (0.1)	-
<i>SLCO1B1</i> _388_G>A (rs2306283)	GG	345 (35.5)	324 (34.5)	1.00 ^b
	GA	479 (49.3)	466 (49.5)	1.03 (0.85-1.26)
	AA	148 (15.2)	150 (16.0)	1.08 (0.82-1.42)
<i>SLCO1B1</i> _463_C>A (rs11045819)	CC	706 (71.2)	703 (71.4)	1.00 ^b
	CA	264 (26.6)	257 (26.1)	0.97 (0.79-1.18)
	AA	22 (2.2)	25 (2.5)	1.10 (0.61-1.99)
<i>SLCO1B1</i> _521_T>C (rs4149056)	TT	700 (71.6)	677 (69.3)	1.00 ^b
	TC	249 (25.5)	273 (27.9)	1.12 (0.92-1.38)

	CC	29 (3.0)	27 (2.8)	0.98 (0.57-1.68)
<i>SLCO1B1</i> _1929_C>A (rs34671512)	CC	880 (88.8)	896 (90.4)	1.00 ^b
	CA	109 (11.0)	92 (9.3)	0.83 (0.61-1.11)
	AA	2 (0.2)	3 (0.3)	-
<i>SLCO1B3</i> _3954_G>A (rs7962265)	GG	302 (30.6)	274 (28.1)	1.00 ^b
	GA	500 (50.6)	505 (51.7)	1.14 (0.93-1.41)
	AA	186 (18.8)	197 (20.2)	1.18 (0.91-1.54)
<i>SLCO1B3</i> _1390_G>A (rs2138334)	GG	198 (20.8)	204 (21.4)	1.00 ^b
	GA	488 (51.2)	499 (52.4)	1.00 (0.79-1.27)
	AA	267 (28.0)	249 (26.2)	0.90 (0.69-1.17)
<i>SLCO1B3</i> _334_G>T (rs4149117)	GG	712 (72.3)	698 (70.6)	1.00 ^b
	GT	255 (25.9)	265 (26.8)	1.08 (0.89-1.33)
	TT	18 (1.8)	25 (2.6)	1.38 (0.74-2.57)
<i>SLCO2B1</i> _8614_A>G (rs2851104)	AA	385 (39.2)	363 (36.8)	1.00 ^b
	AG	449 (45.8)	448 (45.4)	1.06 (0.87-1.29)
	GG	147 (15.0)	175 (17.7)	1.29 (0.99-1.68)
<i>SLCO2B1</i> _5910_T>G (rs11236349)	TT	621 (63.3)	630 (60.4)	1.00 ^b
	TG	313 (31.9)	293 (29.7)	0.92 (0.75-1.12)
	GG	47 (4.8)	62 (6.3)	1.33 (0.89-1.97)
<i>SLCO2B1</i> _4142_A>T (rs2712805)	AA	687 (69.9)	657 (66.6)	1.00 ^b
	AT	262 (26.7)	286 (29.0)	1.16 (0.95-1.42)
	TT	34 (3.5)	43 (4.4)	1.34 (0.84-2.13)
<i>SLCO2B1</i> _2366_G>T (rs2851064)	GG	685 (69.8)	657 (66.6)	1.00 ^b
	GT	263 (26.8)	286 (29.0)	1.16 (0.95-1.41)
	TT	34 (3.5)	43 (4.4)	1.33 (0.84-2.12)
<i>SLCO2B1</i> _1011_A>C (rs4100076)	AA	676 (68.8)	668 (67.8)	1.00 ^b
	AC	276 (28.1)	271 (27.5)	0.98 (0.80-1.20)
	CC	31 (3.2)	46 (4.7)	1.53 (0.96-2.45)
<i>SLCO2B1</i> _71_C>T (rs2851069)	CC	390 (39.7)	370 (37.6)	1.00 ^b
	CT	446 (45.3)	441 (44.8)	1.04 (0.86-1.27)
	TT	147 (15.0)	174 (17.6)	1.27 (0.98-1.66)
<i>SLCO2B1</i> _935_G>A (rs12422149)	GG	779 (79.3)	798 (80.9)	1.00 ^b
	GA	186 (18.9)	174 (17.6)	0.91 (0.72-1.14)
	AA	17 (1.7)	15 (1.5)	0.86 (0.42-1.74)
<i>SLCO2B1</i> _1457_C>T (rs2306168)	CC	932 (95.3)	941 (96.0)	1.00 ^b
	CT	46 (4.7)	39 (4.0)	0.89 (0.58-1.38)
	TT	0	0	-
<i>PXR</i> _25385_C>T (rs3814055)	CC	384 (37.9)	383 (37.6)	1.00 ^b
	CT	497 (49.0)	487 (47.7)	0.98 (0.81-1.19)
	TT	133 (13.1)	150 (14.7)	1.11 (0.84-1.46)
<i>PXR</i> _24381_A>C (rs1523127)	AA	390 (38.5)	386 (37.8)	1.00 ^b
	AC	483 (47.7)	479 (47.0)	1.00 (0.83-1.22)
	CC	140 (13.8)	155 (15.2)	1.09 (0.83-1.43)
<i>PXR</i> _24113_G>A (rs2276706)	GG	400 (39.5)	388 (38.0)	1.00 ^b
	GA	485 (47.8)	482 (47.3)	1.02 (0.84-1.24)
	AA	129 (12.7)	150 (14.7)	1.17 (0.89-1.55)
<i>PXR</i> _252_A>G	AA	467 (46.1)	484 (47.5)	1.00 ^b

(rs1464603)	AG	451 (44.5)	446 (43.8)	0.96 (0.80-1.16)
	GG	95 (9.4)	89 (8.7)	0.86 (0.62-1.19)
<i>PXR_7635_A>G</i> (rs6785049)	AA	391 (38.6)	421 (41.3)	1.00 ^b
	AG	486 (48.0)	471 (46.2)	0.90 (0.74-1.09)
	GG	135 (13.3)	128 (12.6)	0.88 (0.67-1.17)
<i>PXR_8055_C>T</i> (rs2276707)	CC	690 (68.1)	682 (67.0)	1.00 ^b
	CT	292 (28.8)	310 (30.5)	1.08 (0.89-1.31)
	TT	31 (3.1)	26 (2.6)	0.84 (0.49-1.44)
<i>PXR_10799_G>A</i> (rs1054191)	GG	754 (74.4)	767 (75.2)	1.00 ^b
	GA	239 (23.6)	235 (23.0)	0.97 (0.79-1.20)
	AA	20 (2.0)	18 (1.8)	0.87 (0.45-1.70)
<i>PXR_11156_A>C</i> (rs3814057)	AA	703 (69.7)	687 (67.4)	1.00 ^b
	AC	277 (27.4)	308 (30.2)	1.15 (0.94-1.40)
	CC	29 (2.9)	25 (2.4)	0.83 (0.48-1.44)

^aOR conditional on age in 5-year classes, adjusted for menopausal status, family history of breast cancer, use of oral contraceptives, body mass index and smoking

^bReference

Abbreviations: CI = confidence interval, OR = odds ratio