ELSEVIER

European Journal of Cancer 41 (2005) 590–600

European Journal of Cancer

www.ejconline.com

Correlates of high-density mammographic parenchymal patterns by menopausal status in a rural population in Northern Greece

Elena Riza [a,b], Isabel dos Santos Silva [a,*], Bianca De Stavola [a], Nicholas Perry [c], Efi Karadedou-Zafiriadou [d], Dimitrios Linos [e], Dionysios-Dennis Remoundos [f], Athena Linos [b]

[a] Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK
[b] Department of Hygiene and Epidemiology, University of Athens Medical School, Athens, Greece
[c] Breast Assessment Centre, West Wing, St Bartholomew's Hospital, London EC1A 7BE, UK
[d] Department of Radiology, Hippocration Hospital, Thessaloniki, Greece
[e] 1st Surgical Clinic, Diagnostic and Therapeutic Centre of Athens "Hygeia", Athens, Greece
[f] Pre-Registration House Officer, Norfolk and Norwich University Hospital, Colney Lane Colney-Norwich NR4 7UY, UK

Received 7 July 2004; received in revised form 9 November 2004; accepted 14 December 2004
Available online 22 January 2005

Abstract

Reproductive factors affect breast cancer risk, but less is known of their associations with mammographic density and whether these differ by menopausal status. We report on a cross-sectional study of 1946 pre- and 3047 post-menopausal women who joined a breast screening programme in Northern Greece during 1993–1997. The odds of having a high-density Wolfe pattern (P2/DY) was inversely associated with age (*P* for linear trend <0.001) in both pre- and post-menopausal women and, for post-menopausal women, with years since menopause ($P < 0.001$). The odds of a P2/DY pattern declined with higher parity ($P < 0.001$) and younger age at first pregnancy ($P = 0.05$) in both pre- and post-menopausal women. They also decreased with the duration of breast-feeding in pre-menopausal women ($P = 0.03$ in pre- and $P = 0.69$ in post-menopausal women; test for interaction with menopausal status: $P = 0.07$). Age at menarche, age at menopause and the number of miscarriages/abortions were not associated with mammographic density. Age at first pregnancy and parity were strong correlates of mammographic density in pre- and post-menopausal women while duration of breast-feeding appeared to be particularly important in pre-menopausal women.

Keywords: Breast density; Mammographic parenchymal patterns; Wolfe classification; Menopausal status; Reproductive variables; Anthropometric variables; Breast cancer

1. Introduction

The radiographical appearance of the breast depends on the relative amount of fat, connective and epithelial tissues contained in the gland. Mammographic density, as measured by the P2/DY patterns originally defined by Wolfe in Ref. [1] or by the percentage of the total breast composed of radiologically dense tissue [2], is a well-established risk factor for breast cancer. The magnitude of the breast cancer risk associated with increasing mammographic density is approximately a 4- to 6-fold increase when density is measured as percentage of breast density (top versus bottom quintile), and a 2- to 4-fold increase when it is measured indirectly by

* Corresponding author. Tel.: +44 20 7927 2113; fax: +44 20 7580 6897.
E-mail address: isabel.silva@lshtm.ac.uk (I. dos Santos Silva).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2004.12.014

Wolfe patterns (P2/DY versus N1/P1) [2–5]. It has been estimated that having any measurable mammographic density is responsible for 46% of all breast cancer cases in the United States of America (USA), with density of 50% or more accounting for 28% and density of 75% or more accounting for 8% of all cases [2,3].

There is consistent evidence that parity [6–8] is associated with mammographic density, but for other reproductive-related factors, such as age at menarche, age at first birth or age at menopause, the evidence is far from conclusive and, to our knowledge, only one study [9] has so far investigated the association of breast-feeding with mammographic patterns. In addition, the prevalence of high-density mammographic parenchymal patterns is known to change with menopausal status [7], but it is unclear whether correlates of mammographic patterns differ by menopausal status as some studies have reported differential effects of some reproductive-related variables by menopausal status [8,10]. To address these questions, we examined correlates of mammographic parenchymal patterns separately in pre- and post-menopausal women using data from the Ormylia population-based screening programme in Northern Greece. This screening programme covers a traditional rural population characterised by low nulliparity, early age at first birth and high prevalence of breast-feeding, but very low prevalence of ever use of exogenous hormones such as oral contraceptives and hormone replacement therapy [11]. This study population allowed a more detailed investigation of the role of endogenous reproductive factors than had been possible in previous studies, which were conducted in more Westernised populations.

2. Patients and methods

2.1. Study population

The Ormylia Mammography Screening Programme is a population-based breast screening programme set up in 1992, which aimed to provide a mammographic examination every two years to all women aged 40–65 years who are resident in Halkidiki (Northern Greece). This is a predominantly rural region with a total population of approximately 92 000. The coverage of the screening programme was estimated to have been only approximately 60% in 1993/97 when the data analysed here were collected, mainly because of its staggered introduction across the region. At the time of joining the screening programme, every woman completed an interviewer-administered questionnaire on demographic, reproductive and other health-related variables.

Subjects for the present study were all women who had their first (prevalent) mammogram in the screening programme from June 1993 until December 1997. Women with a diagnosis of breast cancer were not eligible. A total of 5017 non-breast cancer women were first screened during this period. Three pre-menopausal women were excluded because their age at mammography was unknown, two because of missing information on Wolfe grade and sixteen because they reported a history of menstrual periods at ages 55 years and over and there were concerns regarding the quality of these data. Similarly, one post-menopausal woman was excluded because age at mammography was unknown and two because of missing information on Wolfe grade. These exclusions left 1946 (39%) pre- and 3047 (61%) post-menopausal women available for the present study.

Each set of mammograms (two views per breast) was read by one of the two programme's radiologists who assigned one of the four Wolfe patterns (N1, P1, P2 or DY) using standard criteria [12]. Strong correlations ($r = 0.86$–0.96) have been found between quantitative measurements of mammographic density from views of the right and left breast and from cranio-caudal and medio-lateral oblique views of the same breast [13]. We found similar strong correlations with the Wolfe classification with reading disagreements between the four views occurring only in a small percentage (less than 5%) of borderline women. These women were assigned the highest of the two possible adjacent Wolfe grades. To assess the reliability of the radiological classification, a sample of 100 women was randomly selected from those who were first screened between 1993 and 1995. The sets of mammograms for these women were read independently by the two programme's radiologists (observers A and B) and by an external radiologist (observer C). To assess intra-observer variation, each of the programme's radiologists, blindly and independently, re-read the 100 sets of mammograms 10–12 weeks after having completed the first reading.

The exposures of interest are age at first mammogram, age at menarche, age at first pregnancy, age at first full-term birth, parity, number of miscarriages/abortions (information was not available separately for each one of these variables), total duration of breast-feeding (cumulative for all live-born children) and self-reported height, weight and body mass index (BMI, calculated as weight (in kg)/height (in m)^2). For post-menopausal women, age at menopause and years since menopause were also investigated. Menopausal status was defined as cessation of menses at least 12 months prior to the time of interview. Age at menopause for women with surgical menopause ($n = 258$) was defined as the date of bilateral oophorectomy/hysterectomy. Years since menopause were calculated by subtracting the date of the last menstrual period (or surgery) from the date at interview.

The study protocol was approved by the relevant ethics committees and informed consent obtained from all the participants.

2.2. Statistics

For the statistical analysis, women were classified as having either a high- (if they were P2 or DY) or a low- (if they were N1 or P1) density Wolfe pattern. *Kappa* coefficients were calculated to measure intra- and inter-observer agreement [14]. *Kappa* (k) represents the level of agreement between independent readings after correction for the amount of agreement expected by chance. The McNemar's χ^2 test with continuity correction [15] was used to assess the evidence of any systematic differences between radiologists or between repeat readings by the same radiologist.

Potential associations between demographic, anthropometric and reproductive variables and the odds of having a high-density Wolfe pattern (P2/DY) were examined separately for pre- and post-menopausal women. Exposure variables (e.g., height and weight) were categorised into fourths based on the observed frequency distribution among the whole study population of pre- or post-menopausal women, or in year bands or units (e.g., age at menarche, age at first pregnancy, number of miscarriages/abortions). Logistic regression was used to estimate crude and adjusted odds ratios (ORs) and their 95% Confidence Intervals (CIs). As exposure data were collected at the time of mammography, these ORs should be interpreted as prevalence ORs. Linear trends across the exposure variable's categories were assessed via likelihood ratio tests [16]. To examine whether the association of a given exposure variable with the odds of high-density patterns was modified by menopausal status, likelihood ratio tests for interaction were computed.

3. Results

Intra-observer agreement between the first and second mammographic reading was very high for the binary classification of Wolfe grade (i.e., P2/DY versus N1/P1): $\kappa = 0.93$ and $\kappa = 0.97$ for observers A and B, respectively (Table 1). Inter-observer agreement was somewhat lower with k ranging from 0.75 to 0.92. There was no indication of systematic differences between readings performed by the three observers, or between the two replicate readings performed by the same observer (Table 1).

The proportions with a P2 or a DY pattern were much higher for pre-menopausal (43% and 17%, respectively) than for post-menopausal women (31% and 3%). Pre-menopausal women with a P2/DY pattern were, on average, slightly younger at the time of their mammogram, but older at the time of their first pregnancy or of their first full-term birth than pre-menopausal N1/P1 women (Table 2). There was no difference in the average age at menarche or in the proportion of nulligravidity between pre-menopausal N1/P1 and P2/DY women, but the latter had, on average, lower parity and had breast-fed for a shorter period. A higher proportion of pre-menopausal P2/DY women reported a previous history of miscarriages or abortions. There was no difference in reported mean height between the two groups,

Table 1
Intra- and inter-observer variability of the binary Wolfe classification: low- (N1/P1) versus high-density (P2/DY) mammographic parenchymal patterns

Observer/reading		No. of sets	Observer A 1st	Observer A 2nd	Observer B 1st	Observer B 2nd
Observer A, 1st	N1/P1	54				
	P2/DY	46				
Observer A, 2nd	N1/P1	57	po[a] 0.97 pc[a] 0.50			
	P2/DY	43	κ[a] 0.93 $P = 0.25$[b]			
Observer B, 1st	N1/P1	52	po 0.96 pc 0.50	po 0.95 pc 0.50		
	P2/DY	48	κ 0.92 $P = 0.62$[b]	κ 0.90 $P = 0.06$[b]		
Observer B, 2nd	N1/P1	53	po 0.95 pc 0.50	po 0.94 pc 0.50	po 0.99 pc 0.50	
	P2/DY	47	κ 0.90 –[c]	κ 0.88 $P = 0.21$[b]	κ 0.97 –[c]	
External 1st[d]	N1/P1	48	po 0.90 pc 0.49	po 0.87 pc 0.49	po 0.92 pc 0.50	po 0.93 pc 0.49
	P2/DY	52	κ 0.80 $P = 0.10$[b]	κ 0.75 $P = 0.05$[b]	κ 0.84 $P = 0.28$[b]	κ 0.86 $P = 0.12$[b]

[a] po: proportion observed, pc: proportion expected on the basis of chance, κ: kappa value.
[b] McNemar's x^2 test.
[c] The number of discordant pairs of readings was too small to produce a valid McNemar's test.
[d] The external observer read the mammograms only once.

Table 2
Characteristics of pre- and post-menopausal women with high- (P2/DY) and low- (N1/P1) density mammographic parenchymal patterns

Variable	Pre-menopausal women ($N = 1946$)						Post-menopausal women ($N = 3047$)					
	P2/DY			N1/P1			P2/DY			N1/P1		
	N	Mean	SD[a]	*N*	Mean	SD[a]	*N*	Mean	SD[a]	*N*	Mean	SD[a]
All	1166			780			1022			2025		
Demographic												
Age at mammography (years)	1166	44.1	4.7	780	44.6	4.9	1022	56.1	6.2	2025	58.7	6.6
Anthropometric												
Reported height (cm)	998	162.6	5.6	668	162.4	5.7	890	161.4	5.7	1763	160.7	5.7
Missing	*168*			*112*			*132*			*262*		
Reported weight (kg)	999	71.6	11.1	667	74.8	12.7	891	72.4	9.8	1767	74.8	10.5
Missing	*167*			*113*			*131*			*258*		
Reported BMI (kg/m^2)	998	27.1	4.1	667	28.4	4.6	890	27.8	3.6	1763	29.0	3.9
Missing	*168*			*113*			*132*			*262*		
Reproductive												
Age at menarche (years)	1081	13.2	1.3	739	13.2	1.3	992	13.5	1.4	1963	13.4	1.5
Missing	*85*			*41*			*30*			*62*		
Age at first pregnancy (years)	1061	21.9	3.5	722	21.6	3.8	930	23.7	4.1	1901	23.5	4.1
Missing	*73*			*37*			*33*			*61*		
Age at first live birth (years)	1041	22.2	3.7	710	22.0	4.0	915	23.9	4.0	1887	23.7	4.1
Missing	*77*			*39*			*32*			*57*		
Total duration of breast-feeding (months)[b,c]	1108	6	0–72	742	7	0–76	939	12	0–96	1927	17	0–192
Missing	*10*			*7*			*8*			*17*		
Never pregnant[d]		2.7%			2.7%			5.8%			3.1%	
Missing		*0.4%*			*0.5%*			*0.5%*			*0.7%*	
With 3+ children[d]		25.0%			30.6%			22.9%			33.1%	
Missing		*0.8%*			*0.6%*			*0.8%*			*0.8%*	
With 1+ miscarriage/abortion[d]		50.7%			49.5%			48.0%			45.0%	
Missing		*0.8%*			*0.6%*			*0.8%*			*0.8%*	
Age at menopause (years)		NA			NA		1016	48.1	4.7	2018	47.8	5.2
Missing							*6*			*7*		
Time since menopause (years)[b]		NA			NA		1016	6	1–41	2018	10	1–45
Missing							*6*			*7*		

BMI = body mass index; NA: not applicable.
[a] Mean and standard deviation (SD), unless otherwise specified.
[b] Median and minimum-maximum.
[c] Cumulative for all live-born children.
[d] Percentage.

but pre-menopausal P2/DY women were, on average, slimmer than pre-menopausal N1/P1 women. Similar relationships were observed among post-menopausal women, except for the proportion of nulligravidity that was higher among P2/DY than N1/P1 women (Table 2). P2/DY women also had a later age at menopause and a shorter time since menopause. Post-menopausal women with a P2/DY pattern were slimmer and, in contrast to their pre-menopausal counterparts, slightly taller than N1/P1 women (Table 2).

Menopausal status was a strong correlate of high-density mammographic patterns, with the odds of having a P2/DY pattern among post-menopausal women being only 34% (OR = 0.34, 95% CI: 0.30, 0.38) of the odds among pre-menopausal women. The magnitude of this association was attenuated, but remained statistically significant, after adjustment for BMI, parity, age at first pregnancy and months of breast-feeding (OR = 0.65, 95% CI: 0.53, 0.78). Age at mammography was inversely associated with the odds of having a P2/DY pattern in pre- and post-menopausal women (*P* for linear trend equal or <0.001 in both), and these associations persisted after adjustment for reproductive and anthropometric variables (Table 3). In post-menopausal women, the odds of having a P2/DY pattern decreased with time since menopause (*P* for linear trend <0.001), but not with age at menopause (Table 3). Further adjustment for age at mammography attenuated only slightly the association with time since menopause (OR for 10+ years *vs.* 1–2 years = 0.49 (95% CI: 0.37, 0.64), *P* for linear trend <0.001).

Mammographic density was inversely associated with weight and BMI at mammography in pre- and post-menopausal women (Table 4), with women in the top fourth of the BMI distribution having only 50–60% of the odds of those in the bottom fourth (*P* for linear trend <0.001). After adjusting for the effect of demographic and reproductive variables, no association with

Table 3
Associations between high-density mammographic parenchymal patterns (P2/DY) and age at mammography, age at menopause and time since menopause: Odds Ratios (OR) and 95% Confidence Intervals (CI)

Variable:	Pre-menopausal women (N = 1946)						Post-menopausal women (N = 3047)					
	N	% P2/DY	Crude		Adjusted[a]		N	% P2/DY	Crude		Adjusted[a]	
			OR	(95% CI)	OR	(95% CI)			OR	(95% CI)	OR	(95% CI)
Categories												
Age (years)												
<45	1002	63	1.58	(1.21, 2.05)	1.49	(1.12, 1.99)	49	47	1.06	(0.59, 1.90)	1.33	(0.72, 2.47)
45–49	648	60	1.41	(1.07, 1.86)	1.40	(1.04, 1.89)	221	46	1.12	(0.82, 1.51)	1.16	(0.84, 1.60)
50–54	296	51	1		1		732	43	1		1	
55–59			–	–	–	–	846	35	0.71	(0.58, 0.87)	0.71	(0.57, 0.88)
60–64			–	–	–	–	698	24	0.41	(0.33, 0.52)	0.41	(0.33, 0.54)
65+			–	–	–	–	501	23	0.38	(0.30, 0.49)	0.35	(0.26, 0.47)
P-value for linear trend[b]			*0.001*		*0.01*				*<0.001*		<0.001	
Age at menopause (years)[c]												
<45			–	–	–	–	632	29	1		1	
45–49			–	–	–	–	1093	37	1.41	(1.14, 1.74)	1.45	(1.16, 1.82)
50–54			–	–	–	–	1132	34	1.26	(1.02, 1.56)	1.31	(1.05, 1.65)
55+			–	–	–	–	177	28	0.94	(0.65, 1.36)	1.10	(0.74, 1.63)
P-value for linear trend[b]			–		–				***0.53***		***0.23***	
Time since menopause (years)[c]												
1–2			–	–	–	–	494	49	1		1	
3–4			–	–	–	–	379	46	0.89	(0.68, 1.17)	0.87	(0.66, 1.16)
5–9			–	–	–	–	773	32	0.49	(0.39, 0.62)	0.46	(0.36, 0.59)
10+			–	–	–	–	1401	25	0.35	(0.28, 0.43)	0.33	(0.26, 0.42)
P-value for linear trend[b]			–		–				*<0.001*		*<0.001*	

[a] ORs adjusted for body mass index (BMI), parity, age at first pregnancy, and months of breast-feeding (all categorical).
[b] Likelihood ratio test for linear trend among the variable's categories.
[c] There were 13 postmenopausal women with missing age at menopause.

adult height was observed in either pre- or post-menopausal women (Table 4).

The odds of having a high-density mammographic pattern were inversely associated with parity in pre- (P for linear trend = 0.05) and post-menopausal (P < 0.001) women (Table 5). Mammographic density was also inversely associated with total duration of breast-feeding, but after adjustment for potential confounders this trend was statistically significant only in pre-menopausal women (P = 0.002). There was a positive association between mammographic density and age at first pregnancy (and age at first full-term birth) in pre- and post-menopausal women, but this association was only statistically significant in post-menopausal women (Table 5). No clear associations were found in either pre- or post-menopausal women between the odds of having a high-density pattern and age at menarche or number of miscarriages/abortions (Table 5).

The variables found to be statistically significantly associated with the odds of a high-density pattern in the previous analyses were included simultaneously in the same model to determine which ones were independent correlates of mammographic density (Table 6). The odds of a high-density pattern increased with increasing age at first pregnancy (P for linear trend = 0.05), but declined with menopausal status/time since menopause, age, parity and BMI at mammography (P for linear trend <0.001 for all these variables). The odds of having a high-density pattern decreased with increasing duration of breast-feeding only in pre-menopausal women (P for linear trend = 0.03), with a borderline significant test for interaction with menopausal status (P = 0.07).

4. Discussion

The main aim of this study was to investigate the role of reproductive-related variables on high-density Wolfe patterns and to assess whether their effects differed by menopausal status. The study was conducted within a population-based screening programme in Northern Greece. This programme covered a rural traditional population in which women had, on average, lower nulliparity, lower age at first birth, higher duration of breast-feeding and lower prevalence of use of exogenous hormones than those in more Westernised populations. The study population was large enough to allow investigation of correlates of high-density mammographic

Table 4
Associations between high-density mammographic parenchymal patterns (P2/DY) and anthropometric measures by menopausal status: Odds Ratios (OR) and 95% Confidence Intervals (CI)

	Pre-menopausal women (N = 1946)[a]						Post-menopausal women (N = 3047)[a]					
Variable:	N	% P2/DY	Crude		Adjusted[b]		N	% P2/DY	Crude		Adjusted[c]	
			OR	(95% CI)	OR	(95% CI)			OR	(95% CI)	OR	(95% CI)
Categories												
Height (fourths; in m)[d]												
<1.58	308	60	1		1		677	31	1		1	
1.58–1.59	100	54	0.78	(0.50, 1.23)	0.81	(0.50, 1.29)	174	40	1.48	(1.05, 2.09)	1.40	(0.96, 2.02)
1.60–1.64	550	60	1.00	(0.76, 1.34)	0.96	(0.70, 1.30)	951	31	1.02	(0.82, 1.26)	0.94	(0.74, 1.18)
1.65+	708	61	1.02	(0.77, 1.34)	1.04	(0.78, 1.38)	851	37	1.34	(1.08, 1.66)	1.11	(0.88, 1.41)
P-value for linear trend[e]			*0.69*		*0.63*				*0.04*		*0.70*	
Weight (fourths; in kg)[d]												
<65	400	67	1		1		451	38	1		1	
65–72	487	61	0.79	(0.60, 1.04)	0.86	(0.64, 1.14)	774	40	1.11	(0.87, 1. 41)	1.11	(0.85, 1.44)
73–79	311	62	0.81	(0.60, 1.11)	0.84	(0.61, 1.16)	612	30	0.72	(0.55, 0. 93)	0.70	(0.53, 0.93)
80+	468	52	0.53	(0.40, 0.70)	0.58	(0.43, 0.77)	821	27	0.62	(0.49, 0.80)	0. 59	(0.45, 0.77)
P-value for linear trend[e]			*<0.001*		*<0.001*				*<0.001*		*<0.001*	
BMI (fourths; in kg/m^2)												
<25.4	542	68	1		1		524	43	1		1	
25.4–27.9	405	60	0.70	(0.53, 0.91)	0.72	(0.54, 0.95)	683	40	0.88	(0.70, 1.11)	0.87	(0.67, 1.12)
28.0–30.8	374	56	0.61	(0.46, 0.80)	0.67	(0.50, 0.89)	710	30	0.57	(0.45, 0.72)	0.58	(0.45, 0.76)
>30.8	344	51	0.49	(0.37, 0.65)	0.53	(0.39, 0.71)	736	23	0.40	(0.31, 0.51)	0.40	(0.31, 0.53)
P-value for linear trend[e]			*<0.001*		*<0.001*				*<0.001*		*<0.001*	

[a] The percentage of missing values were similar for all variables in the table. Pre-menopausal women: 14% for the crude and 19% for the adjusted analyses. Post-menopausal women: 13% for the crud and 18% for the adjusted analysis.
[b] Adjusted for age, parity, age at first pregnancy, and months of breast-feeding (all categorical).
[c] Adjusted for age, parity, age at first pregnancy, months of breast-feeding and years since menopause (all categorical).
[d] The numbers in each fourth differ because of digit preference (e.g., for height there was digit preference for 1.50, 1.55, 1.60, 1.65 and 1.70).
[e] Likelihood ratio test for linear trend among the variable's categories.

patterns separately for pre- and post-menopausal women. In particular, because of the policy to screen women aged 40–65 years, the sample of pre-menopausal women was one of the largest used in epidemiological studies conducted among mammography screening participants.

However, the study had some limitations. The cross-sectional nature of the study design does not allow the establishment of temporal relationships between the exposure variables and mammographic parenchymal patterns. Information on the exposure variables (including data on height and weight at the time of mammography) was collected through interviewer-administered questionnaires conducted at the time the women joined the screening programme. Although self-report and recall of past exposures might have been affected by measurement errors these are unlikely to have been differential as neither the women nor their interviewers were aware of their Wolfe patterns. No information was collected on the use of exogenous hormones, but a previously conducted study within this screening population showed that the prevalence of ever use was very low (only 12% of women reported to have ever taken oral contraceptives, fertility drugs, hormone replacement therapy, or any other hormonal drugs) [11]. The Wolfe classification is known to be subjective, but any outcome misclassification is unlikely to have been differential as the radiologists were kept blind in relation to the characteristics of the women except for their age. Moreover, the *kappa* values observed in the present study for the binary Wolfe classification were comparable to those reported in other studies for inter-observer agreement (from 69% to 87% [17–19]) and intra-observer agreement (from 70% to 92% [19,20]).

A large population-based study [8] found that the effect of age on the odds of high-density Wolfe patterns was modified by menopausal status, the association being positive in pre-menopausal women but negative in post-menopausal women. However, as observed in other studies [21,22], age at mammography in the present study was found to be independently and inversely associated with the odds of having a high-density mammographic pattern, with the odds decreasing steeply with increasing age in both pre- and post-menopausal

Table 5
Associations between high-density mammographic parenchymal patterns (P2/DY) and reproductive variables by menopausal status: Odds Ratios (OR) and 95% Confidence Intervals (CI)

	Pre-menopausal women (N = 1946)[a]						Post-menopausal women (N = 3047)[a]					
Variable:	N	% P2/DY	Crude		Adjusted[b]		N	% P2/DY	Crude		Adjusted[c]	
			OR	(95% CI)	OR	(95% CI)			OR	(95% CI)	OR	(95% CI)
Categories												
Age at menarche (years)												
<13	568	59	1		1		790	31	1		1	
13	585	60	1.04	(0.82, 1.31)	1.03	(0.80, 1.33)	943	34	1.14	(0.93, 1.39)	1.02	(0.81, 1.28)
14	402	59	1.01	(0.78, 1.31)	0.93	(0.70, 1.22)	605	37	1.29	(1.03, 1.61)	1.21	(0.95, 1.55)
15+	265	62	1.13	(0.84, 1.52)	1.11	(0.81, 1.52)	617	34	1.15	(0.92, 1.44)	1.14	(0.89, 1.46)
P-value for linear trend[d]			*0.54*		*0.85*				*0.09*		*0.16*	
Parity												
0	79	61	1		1		156	48	1		1	
1	158	63	1.08	(0.62, 1.89)	0.95	(0.52, 1.73)	260	40	0.72	(0.48, 1.07)	0.58	(0.38, 0.91)
2	1164	62	1.04	(0.65, 1.66)	0.97	(0.58, 1.61)	1703	35	0.59	(0.42, 0.82)	0.50	(0.35, 0.72)
3+	531	55	0.79	(0.49, 1.28)	0.73	(0.43, 1.22)	904	26	0.38	(0.27, 0.53)	0.36	(0.24, 0.52)
P-value for linear trend[d]			*0.04*		*0.05*				*<0.001*		*<0.001*	
No. miscarriages/abortions												
0	955	59	1		1		1591	35	1		1	
1	450	63	1.15	(0.92, 1.45)	1.16	(0.90, 1.49)	738	34	0.95	(0.79, 1.14)	0.98	(0.80, 1.20)
2	291	63	1.18	(0.90, 1.55)	1.18	(0.88, 1.58)	382	32	0.87	(0.68, 1.10)	0.85	(0.65, 1.11)
3+	236	53	0.77	(0.58, 1.03)	0.81	(0.59, 1.11)	312	29	0.77	(0.59, 1.00)	0.86	(0.64, 1.15)
P-value for linear trend[d]			*0.46*		*0.66*				*0.04*		*0.18*	
Age at first pregnancy (years)[e]												
<20	507	57	1		1		401	26	1		1	
20–21	454	58	1.07	(0.83, 1.38)	1.00	(0.76, 1.30)	528	33	1.36	(1.02, 1.81)	1.34	(0.99, 1.83)
22–24	458	63	1.30	(1.00, 1.68)	1.35	(1.02, 1.79)	884	37	1.60	(1.23, 2.08)	1.63	(1.23, 2.17)
25+	364	60	1.15	(0.87, 1.51)	1.11	(0.82, 1.50)	1018	32	1.32	(1.02, 1.71)	1.44	(1.08, 1.91)
P-value for linear trend[d]			*0.13*		*0.15*				*0.08*		*0.02*	
Age at first live birth (years)[f]												
<20	429	57	1		1		332	27	1		1	
20–22	660	60	1.13	(0.88, 1.44)	1.04	(0.80, 1.35)	840	33	1.32	(1.00, 1.75)	1.33	(0.98, 1.81)
23–24	379	62	1.26	(0.95, 1.67)	1.32	(0.97, 1.80)	864	35	1.45	(1.10, 1.92)	1.64	(1.21, 2.24)
25+	283	60	1.13	(0.84, 1.54)	1.08	(0.78, 1.50)	766	33	1.34	(1.01, 1.78)	1.46	(1.07, 2.01)
P-value for linear trend[d]			*0.24*		*0.28*				*0.10*		*0.02*	
Total duration of breast-feeding (months)[f,g]												
0	236	63	1		1		219	34	1		1	
<7	707	63	0.99	(0.73, 1.34)	0.90	(0.64, 1.26)	545	41	1.37	(0.99, 1.90)	1.38	(0.94, 2.02)
7–12	428	60	0.86	(0.62, 1.19)	0.81	(0.57, 1.17)	523	37	1.15	(0.82, 1.60)	1.22	(0.83, 1.80)
13–24	344	57	0.76	(0.54, 1.07)	0.67	(0.46, 0.98)	840	31	0.87	(0.63, 1. 19)	1.06	(0.73, 1.54)
25+	135	48	0.54	(0.35, 0.83)	0.57	(0.36, 0.91)	739	26	0.68	(0.49, 0.94)	1.06	(0.72, 1.56)
P-value for linear trend[d]			*0.001*		*0.002*				*<0.001*		*0.19*	

[a] The percentage of missing values among pre-menopausal women ranged from <1% to 7% for the crude analysis and from 15% to 16% for the adjusted analyses. Missing values for post-menopausal women ranged from <1% to 3% for the crude analysis and from 14% to 16% for the adjusted analyses.
[b] Adjusted for age and BMI (all categorical).
[c] Adjusted for age, BMI and years since menopause (all categorical).
[d] Likelihood ratio test for linear trend among the variable's categories.
[e] Among ever-pregnant women.
[f] Among women who had ever had a live birth.
[g] Cumulative for all live-born children.

Table 6
Multivariable analysis to identify independent correlates of high-density mammographic parenchymal patterns (P2/DY): Odds Ratios (OR) and 95% Confidence Intervals (CI) ($N = 4057$[a])

Variable:	*N*	% P2/DY	OR	(95% CI)	*P-value for linear trend*[b]	Interaction with menopausal status[c]
Categories						
Menopausal status						
Pre-						
Post- since (years):	1576	60	1			
1–2	404	52	0.82	(0.57, 2.18)		
3–4	301	45	0.85	(0.62, 1.15)		
5–9	613	31	0.52	(0.39, 0.70)		
10+	1163	24	0.47	(0.34, 0.64)	*<0.001*	–
Age (years)						
<45	840	61	1.15	(0.89, 1.48)		
45–49	704	57	1.13	(0.90, 1.43)		
50–54	821	47	1			
55–59	693	35	0.88	(0.69, 1.12)		
60–64	585	23	0.62	(0.46, 0.84)		
65+	414	19	0.50	(0.34, 0.72)	*<0.001*	*0.61*
Parity						
0	}370	}48	}1[d]			
1						
2	2434	46	0.90	(0.70, 1.14)		
3+	1253	36	0.69	(0.53, 0.90)	*<0.001*	*0.48*
Age at first pregnancy (years)						
<20	854	44	1			
20–21	926	45	1.11	(0.90, 1.36)		
22–24	1143	46	1.42	(1.17, 1.73)		
25+	1134	39	1.15	(0.94, 1.42)	*0.05*	*0.53*
Total duration of breast-feeding (months)[e]						
Pre-menopausal women						
0	201	65	1			
<7	604	63	0.91	(0.65, 1.28)		
7–12	366	60	0.85	(0.59, 1.22)		
13–24	284	55	0.71	(0.48, 1.04)		
25+	121	48	0.69	(0.43, 1.11)	0.03	
Post-menopausal women						
0	168	34	1			
<7	485	41	1.43	(0.97, 2.10)		
7–12	456	37	1.29	(0.87, 1.90)		
13–24	717	30	1.12	(0.76, 1.64)		
25+	655	26	1.24	(0.83, 1.86)	*0.69*	*0.07*
BMI (in fourths; kg/m^2*)*						
<25.4	995	56	1			
25.4–27.9	1020	47	0.81	(0.67, 0.98)		
28.0–30.8	1019	39	0.62	(0.52, 0.75)		
>30.8	1023	31	0.45	(0.37, 0.55)	*<0.001*	*0.11*

[a] There were missing values for 370 pre- and 566 post-menopausal women.
[b] Likelihood ratio test for linear trend among the variable's categories.
[c] Likelihood ratio test for the interaction between the categorical variable and menopausal status.
[d] The first two categories of parity had to be merged because of collinearity.
[e] Cumulative for all live-born children.

women. In post-menopausal women, the odds of having a high-density mammographic pattern also declined steeply with time since menopause after adjustment for age and other confounding factors. Other investigators have reported that being post-menopausal is inversely associated with the prevalence of high-density mammographic patterns [23,24], or that late age at menopause is associated with reduced mammographic density [10,25]. To our knowledge, only one study [26] examined the effect of time since menopause on high-density patterns, but only as a potential confounder of the association between mammographic patterns and body weight. The negative trend between time since menopause and the odds of having a high-density pattern observed in the present study supports the hypothesis that absence of exposure to ovarian hormones is protective against mammographic density and would agree with findings from the Nurses' Health Study showing a positive correlation between free percentage oestradiol and the percentage of breast density in post-menopausal women [27].

Only 4.7% of the women in this study were nulliparous, much lower than in other Western European countries where nulliparity has been well above 10% for cohorts of women born since 1930 [28,29]. Similarly to other studies [7,8,10,25,30], we found that parity decreased the odds of having a high-density mammographic pattern in pre- and in post-menopausal women. The negative association between parity and mammographic density is consistent with the results from a recent international collaborative re-analysis of data from most epidemiological studies on reproductive factors and breast cancer conducted worldwide [31], which showed a 7% (95% CI: 5.0%, 9.0%) decline in the risk of this tumour for every additional birth among women who had never breast-fed (after adjustment for age, age at first birth and menopausal status). No relationship was found in the present study, and in another [7], between the number of miscarriages/abortions and high-density mammographic patterns, which concurs with the lack of effect of spontaneous and induced abortions on the risk of developing breast cancer reported by the international collaborative re-analysis mentioned above [32].

Our finding, as well as those from other studies [2,9,10,30,33,34], of a positive association between age at first pregnancy (or age at first full-term birth) and high-density patterns in pre- and post-menopausal women is also in the same direction as the effect of age at first birth on breast cancer risk [35]. However, some studies have reported either no association between age at first pregnancy/birth and mammographic density and/or an inverse relationship [23,24]. Interestingly, the mean age at first birth in the study population was lower (22.1 years in pre- and 23.7 years in post-menopausal women) than in most European countries where the mean age at first birth has ranged from 24.0 to 26.5 years for cohorts born since the 1930's [28,29].

In our study, the percentage of parous women who had ever breast-fed was very high (above 90%), with approximately 44% having breast-fed for more than one year. These figures are in sharp contrast with the much lower levels observed in most Westernised populations [29,36]. We found that total cumulative duration of breast-feeding was inversely associated with the odds of having a high-density Wolfe grade, although only in pre-menopausal women. To our knowledge, only one other study [9] has so far investigated the effect of breast-feeding on mammographic features and, contrary to our results, it reported a positive association between having ever-breast-fed and the odds of having a P2/DY pattern among women attending a Breast Screening Unit in the UK (data were not presented by duration of breast-feeding or menopausal status). Our finding of a protective effect from the duration of breast-feeding on mammographic density, albeit only among pre-menopausal women, is consistent with the results from the international collaborative re-analysis described above [31] which found that the risk of breast cancer in parous women decreased by 4.3% (95% CI, 2.9%, 5.8%) for every 12 months of lifetime breast-feeding, after adjustment for age, age at first birth, parity and menopausal status. Breast-feeding may decrease mammographic density and protect against breast cancer by reducing the rate of breast cell proliferation, either by accelerating cell differentiation and/or by suppressing ovulation and thus reducing exposure of the cells to high oestrogen levels [35]. The failure of our study to detect a protective effect for the duration of breast-feeding in post-menopausal women may partly be due to a greater measurement error among these women as they are less likely to accurately recall how long they breast-fed each one of their children.

Age at menarche has been found to be positively associated with high-density mammographic patterns in pre- [24] and post-menopausal [8,33] women in some studies, but others found no association [25,30], while one [10] reported a positive association in pre-menopausal women, but an inverse relationship in post-menopausal women. We found no consistent relationship between age at menarche and high-density mammographic patterns in either pre- or post-menopausal women. However, it is possible that recall errors might have lead to an underestimation of its true effect.

Our results indicate a protective effect of BMI against high-density mammographic patterns in pre- and post-menopausal women. The observed association of BMI with high-density mammographic patterns in pre-menopausal women is similar to those reported by other studies [2,5,7–9,24,26] and is in the same direction as the effect of BMI on breast cancer risk [37,38]. For post-menopausal women, the observed association was also consistent with most studies that have assessed the relationship between BMI and high-density mammographic patterns [2,23,25,26], but not with the effect of BMI on post-menopausal breast cancer risk [37]. The relationship between BMI and mammographic density reflects primarily a relationship of BMI with the amount of fat tissue in the breast rather than a relationship with the amount of fibro-epithelial tissue. There is evidence that patterns of weight gain throughout life, and not just weight at the time of mammography, are important determinants of high-density patterns [39], but, unfortunately, we could not examine this with our data-set. Some studies [26,40] have reported a positive association between adult height and high-density mammographic patterns, the effect being particularly strong in women who are taller than 1.65 m. We found no association between adult height and mammographic patterns in pre- or post-menopausal women, but our study population was much shorter than those in other studies. Moreover, self-reported height is likely to have been affected by measurement error.

Identification of correlates of mammographic density will improve our understanding of the biological mechanisms underlying breast cancer aetiology. This is particularly important as mammographic density is increasingly being used as a surrogate endpoint for breast cancer in many epidemiological studies [41]. In this study, high-density mammographic patterns were found to be inversely associated with age, parity and BMI at mammography, but positively associated with age at first pregnancy (or first full-term birth), in both pre- and post-menopausal women. In addition, this study was the first to report a negative association between mammographic density and total cumulative duration of breast-feeding which appeared to be particularly important in pre-menopausal women.

Conflict of interest statement

None declared.

Acknowledgement

The authors thank all members of staff at the Centre "Panagia Filanthropini" in Ormylia-Halkidiki for their cooperation and support of this project.

References

1. Wolfe JN. Breast patterns as an index of risk for developing breast cancer. *Am J Roentgenol* 1976, **126**, 1130–1137.
2. Byrne C, Schairer C, Wolfe JN, *et al*. Mammographic features and breast cancer risk: effects with time, age and menopause status. *J Natl Cancer Inst* 1995, **87**, 1622–1629.
3. Byrne C. Studying mammographic density: implications for understanding breast cancer. *J Natl Cancer Inst* 1997, **89**, 531–533.
4. Sala E, Warren R, McCann J, *et al*. Mammographic parenchymal patterns and mode of detection: implications for the breast screening programme. *J Med Screen* 1998, **5**, 207–212.
5. Salminen T, Saarenmaa I, Heikkeila M, *et al*. Risk of breast cancer and changes in mammographic parenchymal patterns. *Acta Oncol* 1998, **37**, 547–551.
6. Sala E, Warren R, McCann J, *et al*. High-risk mammographic parenchymal patterns, hormone replacement therapy and other risk factors: a case–control study. *Int J Epidemiol* 2000, **29**, 629–636.
7. Salminen T, Hakama M, Heikkila M, *et al*. Favorable change in mammographic parenchymal patterns and breast cancer risk factors. *Int J Cancer* 1998, **78**, 410–414.
8. De Stavola B, Gravelle IH, Wang DY, *et al*. Relationship of mammographic parenchymal patterns with breast cancer risk factors and risk of breast cancer in a prospective study. *Int J Epidemiol* 1990, **19**, 247–254.
9. Leinster SJ, Walsh PV, Whitehouse GH, *et al*. Factors associated with mammographic parenchymal patterns. *Clin Radiol* 1988, **39**, 252–256.
10. Gram IT, Funkhouser E, Tabar L. Reproductive and menstrual factors in relation to mammographic parenchymal patterns among perimenopausal women. *Br J Cancer* 1995, **75**, 647–650.
11. Riza E, dos Santos Silva I, De Stavola B, *et al*. Urinary oestrogen metabolites and mammographical parenchymal patterns in post-menopausal women. *Cancer Epidemiol Biomark Prev* 2002, **10**, 627–634.
12. Whitehead J, Carlile T, Kopecky KJ, *et al*. The relationship between Wolfe's classification of mammograms, accepted breast cancer risk factors and the incidence of breast cancer. *Am J Epidemiol* 1985, **122**, 994–1006.
13. Byng JW, Boyd NF, Lockwood G, *et al*. Symmetry of projection in the quantitative analysis of mammographic densities. *Eur J Cancer Prev* 1996, **5**, 319–327.
14. Fleiss JL. *Statistical methods for rates and proportions*. New York, Wiley, 1981.
15. Mc Nemar Q. *Psychological statistics*. 4th ed. New York, Wiley, 1969.
16. Clayton D, Hills M. *Statistical models in epidemiology*. Oxford, Oxford University Press, 1993., p. 261–281.
17. Brisson J, Merletti F, Sadowsky NL, *et al*. Mammographic features of the breast and breast cancer risk. *Am J Epidemiol* 1982, **115**, 428–437.
18. Carlile T, Thompson DJ, Kopecky KJ, *et al*. Reproducibility and consistency in the classification of breast parenchymal patterns. *Am J Roentgenol* 1983, **140**, 1–7.
19. Toniolo P, Bleich AR, Beinart C, *et al*. Reproducibility of Wolfe's classification of mammographic parenchymal patterns. *Prev Med* 1992, **21**, 1–7.
20. Boyd NF, Wolfson C, Moskowitz, *et al*. Observer variation in the classification of mammographic parenchymal patterns. *J Chron Dis* 1986, **39**, 465–472.
21. Wolfe JN. Breast parenchymal patterns and their change with age. *Radiology* 1976, **121**, 545–552.
22. Boyd N, Martin L, Stone J, *et al*. A longitudinal study of the effects of menopause on mammographic features. *Cancer Epidemiol Biomarkers Prev* 2002, **11**, 1048–1053.
23. Bartow SA, Pathak DR, Mettler FA, *et al*. Breast mammographic pattern: a concatenation of confounding and breast cancer risk factors. *Am J Epidemiol* 1995, **142**, 813–819.
24. Salminen T, Saarenmaa I, Heikkeila M, *et al*. Unfavourable change in mammographic patterns and the breast cancer risk factors. *Breast Cancer Res Treat* 1999, **57**, 165–173.
25. Carlile T, Kopecky KJ, Thompson DJ, *et al*. Breast cancer prediction and the Wolfe classification of mammograms. *J Am Med Ass* 1985, **254**, 1050–1053.
26. Gram IT, Funkhouser E, Tabar L. Anthropometric indices in relation to mammographic patterns in peri-menopausal women. *Int J Cancer* 1997, **73**, 323–326.
27. Byrne C, Hankinson S, Golditz G, *et al*. A cross-sectional study of mammographic density and plasma hormones. *Am J Epidemiol* 1999, **149**, 190.
28. Coleman D. Britain in Europe: international and regional comparisons of fertility levels and trends. In Ni Bhrolchain M, ed. *New perspectives on fertility in Britain. Studies on medical and population subjects*, vol. 55. London, HMSO, 1993. pp. 67–93.
29. Swerdlow AJ, dos Santos Silva I, Doll R. *Cancer incidence and mortality in England and Wales. Trends and risk factors*. Oxford, Oxford University Press, 2001.

30. Bergkvist I, Tabar L, Bergstrom R, *et al*. Epidemiologic determinants of the mammographic parenchymal pattern. *Am J Epidemiol* 1987, **126**, 1075–1081.
31. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breast-feeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. *Lancet* 2002, **360**, 187–195.
32. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and abortion: collaborative reanalysis of data from 53 epidemiological studies, including 83000 women with breast cancer from 16 countries. *Lancet* 2004, **363**, 1007–1016.
33. Gravelle IH, Bulstrode RD, Bulbrook RD, *et al*. A prospective study of mammographic parenchymal patterns and risk of breast cancer. *Br J Radiol* 1986, **59**, 487–491.
34. Funkhouser E, Waterbor JW, Cole P, *et al*. Mammographic patterns and breast cancer risk factors among women having elective screening. *South Med J* 1993, **86**, 177–180.
35. Kelsey J. Breast cancer epidemiology: summary and future directions. *Epidemiol Rev* 1993, **15**, 256–263.
36. United States Department of Health and Human Services. Racial and educational factors associated with breast-feeding – United States, 1969–80. *Morb Mortal Weekly Rep* 1984, **33**, 153–154.
37. Hunter DJ, Willett WC. Diet, body size and breast cancer risk. *Epidemiol Rev* 1993, **15**, 110–132.
38. Ursin G, Longnecker MP, Hale RW, *et al*. A meta-analysis of body mass index and risk of pre-menopausal breast cancer. *Epidemiology* 1995, **6**, 137–141.
39. McCormack VA, dos Santos Silva I, De Stavola BL, *et al*. Life-course body size and perimenopausal mammographic parenchymal patterns. *Br J Cancer* 2003, **89**, 852–859.
40. Beijerinck D, van Noord PAH, Seidell JC, *et al*. Abdominal fat predominance in women associated with a decreased incidence of the high risk P2, DY mammographic breast patterns. *Int J Obes* 1991, **15**, 89–93.
41. Cuzick J, Warwick J, Pinney E, *et al*. Tamoxifen and breast density in women at increased risk of breast cancer. *J Natl Cancer Inst* 2004, **96**, 621–628.