

## Obstetric history and mammographic density: a population-based cross-sectional study in Spain (DDM-Spain)

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**Abstract** High mammographic density (MD) is used as a phenotype risk marker for developing breast cancer. During pregnancy and lactation the breast attains full development, with a cellular-proliferation followed by a lobular-differentiation stage. This study investigates the influence of obstetric factors on MD among pre- and post-menopausal women. We enrolled 3,574 women aged 45–68 years who were participating in breast cancer screening programmes in seven screening centers. To measure MD, blind anonymous readings were taken by an experienced radiologist, using craniocaudal mammography and Boyd's

semiquantitative scale. Demographic and reproductive data were directly surveyed by purpose-trained staff at the date of screening. The association between MD and obstetric variables was quantified by ordinal logistic regression, with screening centre introduced as a random effect term. We adjusted for age, number of children and body mass index, and stratified by menopausal status. Parity was inversely associated with density, the probability of having high MD decreased by 16% for each new birth ( $P$  value < 0.001). Among parous women, a positive association was detected with duration of lactation [ $>9$  months: odds ratio (OR) = 1.33; 95% confidence interval (CI) = 1.02–1.72] and weight of first child ( $>3,500$  g: OR = 1.32; 95% CI =

The other members of DDM-Spain are listed in Appendix.

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1.12–1.54). Age at first birth showed a different effect in pre- and post-menopausal women ( $P$  value for interaction = 0.030). No association was found among pre-menopausal women. However, in post-menopausal women the probability of having high MD increased in women who had their first child after the age of 30 (OR = 1.53; 95% CI = 1.17–2.00). A higher risk associated with birth of twins was also mainly observed in post-menopausal women (OR = 2.02; 95% CI = 1.18–3.46). Our study shows a greater prevalence of high MD in mothers of advanced age at first birth, those who had twins, those who have breastfed for longer periods, and mothers whose first child had an elevated birth weight. These results suggest the influence of hormones and growth factors over the proliferative activity of the mammary gland.

**Keywords** Mammographic density · Obstetric history · Age at first birth · Lactation

### Abbreviations

BMI	Body mass index
DDM-Spain	Determinants of mammographic density in Spain
MD	Mammographic density
OR	Odds ratio
95% CI	95% Confidence interval

### Introduction

Mammographic density (MD), i.e., percentage of radiologically dense breast tissue, is one of the strongest known risk factors for breast cancer. This risk is four to five times greater among women with density in more than 75% of the breast compared to women with little or no density [1–3].

During full-term pregnancy and lactation, the breast attains full development due to an early growth stage followed by a subsequent stage of lobular differentiation marked by a change of type 1 to types 3 and 4, resulting in protection of this organ from chemically induced carcinogenesis [4, 5]. These endocrinological and physiological changes are the result of complex interactions among hormones and growth factors. These mitogens, such as oestrogen, prolactin, and IGF-1, modify the tissue composition of the breast, resulting in variations in the mammographic image [6, 7].

Women's obstetric and reproductive history could thus logically be expected to be one of the factors that could modulate MD. Whereas previous studies have reported positive associations between MD and older age at first full-term birth and nulliparity [1, 8–12], the influence of

other factors, such as duration of breastfeeding, miscarriages, birth of twins, and newborns' characteristics, remains uncertain. Some studies have reported differential effects of reproductive variables according to menopausal status, suggesting different susceptibility to endogenous and exogenous factors, and to oestrogenic compounds in particular [10, 12]. Accordingly, this study sought to investigate the influence of reproductive factors on MD among a group of more than 3,500 Spanish women, and to assess whether such effects differed by menopausal status.

### Materials and methods

The DDM-Spain study (Determinantes de la Densidad Mamográfica en España—Determinants of Mammographic Density in Spain) is a cross-sectional multicentre study based on 3,584 women, aged 45–68 years, recruited from specific screening centres within the Spanish Breast Cancer Screening Programme network in the following Spanish Autonomous Regions (Comunidades Autónomas): Aragon; Balearic Isles; Castile-León; Catalonia; Galicia; Navarre; and Valencia. A minimum of 500 women per screening centre were recruited from 7 Oct 2007 to 14 July 2008. Women previously diagnosed with breast cancer or some other malignant disease (except non-melanoma skin cancers) were excluded, as were women unable to respond to the questionnaire and those with any physical problem that prevented a screening mammogram from being performed. Women were contacted by telephone and invited to participate in the study. Those who agreed to participate were given an appointment with the interviewer at the screening centre on the same day as that scheduled for their mammogram, to answer the study questionnaire. Participation was formalised by subjects signing an informed consent document which, among other things, included information on their statutory rights to data-confidentiality and -protection. Participants were allocated an alphanumeric code, consisting of a single letter denoting the particular centre, followed by their respective number.

Each participant was required to provide access to the craniocaudal mammogram of her left breast. To measure MD, we used the Boyd semi-quantitative scale, which classifies density into one of six categories, namely A (0%), B (1–10%), C (11–25%), D (26–50%), E (51–75%), or F (>75%). Blind, anonymous readings were taken by a single, experienced radiologist. By way of quality control, a concordance study was undertaken on a subsample and showed a high concordance between the first and second readings (weighted kappa values of 0.92) [13]. Purpose-trained interviewers administered a structured questionnaire, which recorded demographic data, as well as data on childhood and youth, family and personal background,

gynaecological, obstetric and occupational history, domestic activities and lifestyle. Women's height and weight were measured twice by the interviewer in accordance with standardised protocols, with a third measurement being taken in cases where the first two were dissimilar. Average values were used to compute body mass index (BMI). With respect to obstetric history, the questionnaire collected information on fertility problems and their treatment, number of miscarriages and, in the case of women who had borne children, the number of children, newborns' sex and weight, year of birth, type of gestation, and duration of maternal lactation.

MD was included as an ordinal response variable, and its association with all the variables of interest was evaluated by using ordinal logistic models with random screening centre-specific intercepts [14]. Ordinal logistic regression, also known as the proportional-odds model, assumes that odds ratios (ORs) remain constant, irrespective of the cut-off chosen to dichotomize the ordinal classification of MD in two groups: high versus low MD. The model simultaneously estimates as many equations as the number of categories in the dependent variable minus one. The Brant test was used to verify this *proportional odds assumption*. Due to the few women belonging to category A (4.2%) and F (5.3%) both were combined with the adjacent group. Hence, all logistic models included MD as an ordinal response variable with four categories. The main exposure variables (reproductive characteristics), as well as the remaining adjustment factors, were deemed to be fixed effects, so that their associated ORs were constrained to be the same for women at all screening centres. The models also included a random centre-specific intercept term, which accounted for unexplained variations in the baseline ORs of higher MD across screening centres, as well as known strong determinants of MD, including age at mammography, BMI, number of children, and menopausal status.

In the next step, a global model was fitted in the subgroup of parous women, simultaneously including all reproductive variables that were associated with MD (number of children, age at first full-term birth, birth of twins, lactation, and weight of first child) and adjusting for age and BMI. This analysis was repeated, taking pre and post-menopausal women separately.

All analyses were performed in Stata (StataCorp LP, College Station, TX), using the `gllamm` function to fit random-intercept ordinal logistic models [15].

## Results

Three thousand, five hundred and eighty-four (3,584) women were recruited and interviewed. The average participation rate was 74.5%, ranging from 64.7% at the

Corunna (Galicia) to 84.0% at the Zaragoza screening centre (Aragon). MD assessment was completed for 3,567 participants. All women who developed breast cancer within 6 months of mammography screening ( $n = 10$ ) were excluded from the analysis.

Table 1 shows the distribution of certain socio-demographic factors and reproductive variables by Boyd scale grade, along with their ORs and 95% confidence intervals (CI), adjusted for age, BMI, menopausal status, and number of children. There was a positive association with age at first birth ( $>29$  years: OR = 1.28; 95% CI = 1.04–1.58), with a significant trend in evidence ( $P$  value = 0.039). An inverse association with the number of children was also detected, whereby the OR of having higher MD decreased by 20% for each new birth ( $P$  value  $< 0.001$ ). Among women with children, there was a greater prevalence of high MD among those who had borne twins (OR = 1.72; 95% CI = 1.10–2.68) and those whose first child's birth weight exceeded 3,500 g (OR = 1.30; 95% CI = 1.11–1.53); indeed, in the latter case, risk rose by 4% for every 250 g increase in the weight of the newborn ( $P$  value = 0.006). This same association, albeit somewhat more attenuated, was likewise observed in relation with the average weight of all the children born of any given woman.

Finally, the risk of having higher MD was also seen to increase with duration of maternal lactation: women who breastfed their first child for more than 9 months registered a 38% increase in the odds of being in high MD categories (OR = 1.38; 95% CI = 1.05–1.82), with a statistically significant trend ( $P$  value = 0.003). On examining this result in depth, we decided to calculate each woman's cumulative lifetime lactation. Risk was observed to rise until 18 months and fall off slightly thereafter among women with the greatest cumulative lactation time. As there was a strong correlation between duration of breastfeeding after first birth and cumulative duration of breastfeeding (Spearman coefficient = 0.85,  $P$  value  $< 0.0001$ ), only one of these was used in subsequent analyses. Duration of breast feeding after first birth was chosen, as it was better reported and did not depend on the number of children.

Table 2 shows the joint analysis of reproductive variables for all women who had children, stratified by menopausal status and additionally adjusted for the above-mentioned variables, which, in some cases (i.e., breastfeeding duration) were recoded, with categories having similar risks being pooled. In general, the estimators obtained in this second model were similar to those shown in Table 1. The protective effect associated with the number of children was in evidence in both pre- and post-menopausal women, though it only reached statistical significance in the latter ( $>4$  children: OR = 0.50; 95% CI = 0.31–0.80). Age at first birth, however, displayed different effects in pre- and post-menopausal women, with the interaction term proving statistically

**Table 1** Characteristics of the study population by Boyd grade, and associated ORs for higher Boyd grade

	N	Mammographic density (%)						OR <sup>b</sup>	95% CI	P value <sup>c</sup>
		0	<10	11–25	26–50	51–75	>75			
Reproductive variables										
Fertility problems										
No	3,323	4	21	21	32	17	5	1.00		
Yes with treatment	105	3	17	23	32	17	8	0.86	0.60–1.24	0.427
Yes without treatment	128	5	16	16	29	27	9	1.21	0.86–1.70	0.272
Age at first birth										
Nulliparous	318	4	11	15	35	23	12	1.27	0.95–1.72	0.111
<20	155	9	17	30	23	16	5	1.04	0.76–1.41	0.824
20–24	1,347	5	24	23	30	15	4	1.00		
25–29	1,271	4	21	20	35	16	5	1.11	0.96–1.28	0.162
>29	465	4	15	17	31	27	6	1.28	1.04–1.58	0.021
Two-weekly trend								1.09	1.00–1.19	0.039
No. of children										
None without miscarriages	277	4	10	16	34	24	13	1.76	1.38–2.25	<0.001
None with miscarriages	41	5	15	7	44	20	10	1.61	0.90–2.86	0.107
1	543	2	15	17	33	27	6	1.35	1.13–1.63	0.001
2	1,708	4	20	20	33	17	5	1.00		
3	715	5	24	24	29	14	4	0.94	0.80–1.11	0.484
4	189	9	28	28	30	4	2	0.68	0.51–0.90	0.007
>4	83	14	39	24	19	1	2	0.47	0.31–0.72	0.001
Trend								0.80	0.76–0.85	<0.001
Birth of twins <sup>a</sup>										
No	3,169	4	21	21	32	17	5	1.00		
Yes	69	3	14	19	35	22	7	1.72	1.10–2.68	0.017
Lactation first child (months) <sup>a</sup>										
<1 Month	954	3	25	21	30	15	5	1.00		
1–3	935	5	20	22	32	17	4	1.00	0.84–1.18	0.956
4–6	596	4	19	21	34	18	4	1.18	0.97–1.43	0.093
7–9	232	3	17	19	33	21	6	1.33	1.01–1.74	0.040
>9	226	5	22	20	33	17	3	1.38	1.05–1.82	0.021
Three-monthly trend								1.07	1.02–1.12	0.003
Cumulative lactation in all children <sup>a</sup>										
<1 Month	817	4	25	19	30	17	5	1.00	0.84–1.19	0.989
1–6	1,080	4	18	23	33	17	5	1.00		
7–12	690	4	19	20	31	20	5	1.17	0.98–1.40	0.084
13–18	290	4	19	19	38	17	3	1.33	1.04–1.70	0.021
>18	354	7	27	22	30	10	4	1.19	0.93–1.51	0.160
Three-monthly trend								1.02	1.01–1.04	0.013
Time since weaned (years) <sup>a</sup>										
0–15	289	2	14	11	33	30	10	1.13	0.84–1.52	0.437
16–20	430	2	14	22	30	26	7	1.11	0.87–1.41	0.400
21–25	716	3	18	22	33	18	5	1.00	0.83–1.21	0.978
26–30	911	5	22	22	33	14	5	1.00		
31–35	648	7	28	21	30	12	2	0.90	0.74–1.10	0.306
>35	235	6	30	26	29	8	1	0.83	0.62–1.10	0.191
Two-weekly trend								0.94	0.88–1.01	0.095

**Table 1** continued

	N	Mammographic density (%)						OR <sup>b</sup>	95% CI	P value <sup>c</sup>
		0	<10	11–25	26–50	51–75	>75			
<b>Weight of first child (g)<sup>a</sup></b>										
≤3,500	778	5	22	19	31	18	5	0.94	0.79–1.11	0.440
3001–3,500	1,288	4	21	23	33	17	3	1.00		
>3,500	873	4	21	21	31	18	5	1.30	1.11–1.53	0.001
Trend by 250 g								<i>1.04</i>	<i>1.01–1.08</i>	<i>0.006</i>
<b>Average weight of all children<sup>a</sup></b>										
≤3,000	707	4	21	18	31	19	6	0.98	0.83–1.16	0.800
3,001–3,500	1,388	4	19	22	33	17	4	1.00		
>3,500	1,121	4	24	21	31	16	5	1.14	0.98–1.32	0.084
Trend by 250 g								<i>1.03</i>	<i>1.00–1.07</i>	<i>0.048</i>
<b>Other determinants of MD</b>										
<b>Age (years)</b>										
<50	541	1	9	13	35	31	11	1.00		
50–54	979	2	17	19	33	22	7	0.87	0.69–1.10	0.229
55–59	1,002	5	20	25	32	13	4	0.76	0.59–0.99	0.039
>59	1,035	7	30	22	29	10	2	0.53	0.41–0.70	<0.001
Two-weekly trend								<i>0.79</i>	<i>0.73–0.85</i>	<i>&lt;0.001</i>
<b>BMI</b>										
<20	66	0	9	8	23	39	21	2.45	1.44–4.16	0.001
20–24	950	1	10	15	35	28	11	1.00		
25–29	1,482	3	19	22	34	18	4	0.55	0.47–0.64	<0.001
≥30	1,044	10	32	24	27	6	2	0.22	0.18–0.26	<0.001
Trend								<i>0.55</i>	<i>0.50–0.59</i>	<i>&lt;0.001</i>
<b>Current situation with respect to menstrual period</b>										
No longer has period	2,751	5	23	23	32	14	4	1.00		
Period with menstrual irregularities	381	2	14	17	31	28	8	1.38	1.10–1.72	0.006
Still has period	423	0	11	10	34	33	11	1.84	1.46–2.33	<0.001

95% CI confidence interval

<sup>a</sup> Nulliparous women excluded<sup>b</sup> ORs adjusted for age, BMI, menopausal status, and number of children. Italic figures refer to ORs and 95% CI obtained using the continuous variable without categorization<sup>c</sup> P value. In italic those obtained with the variable as a continuous term

significant ( $P$  value = 0.030): while no association with this variable was found among pre-menopausal women, in post-menopausal women the OR rose by 18% for every 5-year increase in age at first birth, so that post-menopausal women who had given birth to their first child after the age of 30 registered a 53% excess risk of being classified in categories of higher MD (OR = 1.53; 95% CI = 1.17–2.01). The above-described positive association with birth of twins was confirmed mainly in post-menopausal women (OR = 1.88; 95% CI = 1.10–3.23). Although duration of maternal lactation displayed a positive association with density, showing a growing trend in pre- and post-menopausal women alike, once again it was only in the latter that this proved to be statistically significant (OR = 1.07 for every 3 months of lactation). Finally, elevated birth weight of the first child was

associated with a greater prevalence of high MD in both groups, though in this particular case the association was stronger among pre-menopausal women (>3,500 g: OR = 2.05; 95% CI = 1.41–2.98).

The analyses were repeated including still births in the number of children, but this led to no change in the results (data not shown).

## Discussion

This article examines the association between certain reproductive variables and MD in a sample of Spanish women who were participating in a breast cancer screening programme, and assesses whether the observed effects

**Table 2** ORs, 95% confidence intervals and P values for higher Boyd grade associated with characteristics of the study population, by menopausal status

	All women (N = 3238)				Pre-menopausal women (N = 720) <sup>e</sup>				Post-menopausal women (N = 2516) <sup>e</sup>			
	N	OR <sup>b</sup>	95% CI	P <sup>c</sup>	N	OR <sup>b</sup>	95% CI	P <sup>c</sup>	N	OR <sup>b</sup>	95% CI	P <sup>c</sup>
<b>No. of children<sup>d</sup></b>												
1	543	1.29	1.05–1.58	0.015	171	1.28	0.86–1.91	0.217	372	1.30	1.02–1.65	0.032
2	1,708	1.00			408	1.00			1298	1.00		
3	715	1.00	0.84–1.019	0.998	113	0.88	0.57–1.34	0.537	602	1.02	0.84–1.24	0.817
4	189	0.66	0.49–0.88	0.006	28	0.56	0.25–1.28	0.169	169	0.68	0.50–0.94	0.018
>4	83	0.51	0.32–0.80	0.003					75	0.50	0.31–0.80	0.004
Trend		<i>0.84</i>	<i>0.77–0.91</i>	<i>&lt;0.001</i>		<i>0.84</i>	<i>0.69–1.02</i>	<i>0.071</i>		<i>0.84</i>	<i>0.77–0.91</i>	<i>&lt;0.001</i>
<b>Age at first birth</b>												
<20	155	1.00	0.72–1.37	0.979	43	1.29	0.66–2.52	0.451	112	0.90	0.62–1.30	0.581
20–24	1,347	1.00			281	1.00			1065	1.00		
25–29	1,271	1.13	0.97–1.32	0.104	243	0.99	0.69–1.40	0.934	1027	1.16	0.98–1.37	0.093
>29	465	1.34	1.06–1.69	0.013	153	0.92	0.59–1.46	0.734	312	1.53	1.17–2.01	0.002
Two-weekly increase		<i>1.12</i>	<i>1.02–1.22</i>	<i>0.020</i>		<i>0.94</i>	<i>0.78–1.12</i>	<i>0.471</i>		<i>1.18</i>	<i>1.06–1.32</i>	<i>0.002</i>
<b>Birth of twins</b>												
No	3,169	1.00			706	1.00			2461	1.00		
Yes	69	1.74	1.08–2.83	0.024	14	1.32	0.43–4.09	0.629	55	1.88	1.10–3.23	0.021
<b>Lactation of first child (months)<sup>a</sup></b>												
<4	1,889	1.00			425	1.00			1463	1.00		
4–6	596	1.13	0.95–1.34	0.175	122	1.21	0.81–1.79	0.356	474	1.12	0.92–1.36	0.259
7–9	232	1.28	0.99–1.66	0.057	98	1.28	0.82–1.98	0.276	171	1.30	0.96–1.74	0.085
>9	226	1.36	1.04–1.77	0.022					188	1.37	1.03–1.83	0.033
Three-monthly increase		<i>1.06</i>	<i>1.01–1.11</i>	<i>0.011</i>		<i>1.04</i>	<i>0.94–1.15</i>	<i>0.476</i>		<i>1.07</i>	<i>1.02–1.13</i>	<i>0.010</i>
<b>Weight of first child (g)</b>												
≤3,000	778	0.92	0.78–1.09	0.346	189	1.17	0.81–1.67	0.402	588	0.86	0.71–1.04	0.124
3,001–3,500	1,288	1.00			281	1.00			1007	1.00		
>3,500	873	1.31	1.11–1.53	0.001	174	2.05	1.41–2.98	<0.001	698	1.17	0.98–1.40	0.085
Increase by 250 g		<i>1.05</i>	<i>1.02–1.06</i>	<i>0.004</i>		<i>1.08</i>	<i>1.01–1.17</i>	<i>0.034</i>		<i>1.04</i>	<i>1.01–1.06</i>	<i>0.024</i>

95% CI confidence interval

<sup>a</sup> In the group of pre-menopausal women the categories of 7–9 and >9 months were pooled due to the low number of cases<sup>b</sup> ORs adjusted for age, BMI and the remaining variables shown in the table. Italic figures refer to ORs and 95% CI obtained using the continuous variable without categorization<sup>c</sup> P value. In italic P value obtained with the variable as a continuous term<sup>d</sup> In the group of pre-menopausal women the categories of 4 and >4 children were pooled due to the low number of cases<sup>e</sup> There were two women who failed to report their menopausal status

differ in terms of menopausal status. Our results show a protective effect associated with parity. Among women who have borne children, there is a greater risk of high MD associated with the birth weight of the first child, and this is in evidence among pre-menopausal women in particular. In post-menopausal women who have given birth, there is a positive relationship between mammographic density and age at first birth, birth of twins and duration of lactation.

One of the main advantages of our study lies in the large size and population-based nature of the study sample. It is the largest epidemiological study to have analysed the association between MD and breast cancer risk factors in the Spanish

population. The women recruited were attending the corresponding breast cancer screening centres to which all Spanish women coming within the age range defined by their respective regional programmes are invited. The average participation rate was 74.5%, and ranged from 64.7% in Corunna (Galicia) to 84.0% in Zaragoza (Aragon). Density measurements were made by a single experienced radiologist and showed a high degree of internal concordance [13]. In addition, the ordinal nature of the dependent variable was taken into account, by using ordinal logistic regression rather than traditional logistic regression models, which entail a loss of valuable information by combining different MD categories.

Our study also has a series of limitations. First, it is a cross-sectional study, which means that the effect of changes in density patterns cannot be investigated. Second, the explanatory variables of interest are self-reported and so subject to the influence of possible recall bias. However, since density assessment was blind and anonymous, any recall bias would not be differential, thus implying an underestimate of the effects studied. Furthermore, our sample corresponds to the screening programme target population (women aged 45–68 years), so that the number of pre-menopausal women might be insufficient for the purpose of detecting significant differences in some associations. Finally, measurement of density was performed by a highly experienced radiologist using a categorical scale with a high degree of internal consistency. The use of quantitative methods for measurement of MD is frequent in the literature [16, 17]. Even so, such methods are not free of subjectivity and have been validated solely for mammograms taken by analogue mammography machines. In our study, 3 of the 7 participant centres used digital images.

Parity, or having a greater number of children, has been inversely associated with MD in many previous studies [1, 7, 8, 10–12, 18]. In our study, we detected that the OR decreased by a mean of 16% per child. Various authors have postulated that the reduction in MD with age and menopause reflects the process of involution of mammary tissue [19–21], and have even gone so far as to show an inverse association between mammary density percentage and the process of involution [19]. In this process, the glandular epithelium is initially replaced by stroma and, with time, the stroma is then replaced by fat. Completely involuted tissue is thus made up of atrophic epithelium and fat, and would therefore have little MD [19]. Nevertheless, the relationship between involution and MD seems to be more complex, as is highlighted by the fact that an inverse association between mammary involution and parity has been described [22]. Whereas the authors of this study suggest that the relationship between parity and breast cancer may not be mediated by mammary involution, other authors contend that the state of involution may in part depend on parity because, after successive pregnancies, stem and/or progenitor cells accumulate in the mammary glands of multigestational female mice [23], a valid hypothesis for also explaining the relationship between density and parity.

Another factor that was studied is the age at which women have their first child, which showed a positive association with MD in postmenopausal women. Other authors have also reported a positive association, though the results are less consistent than in the case of parity [10–12, 18, 24]. Full-term pregnancy induces a change in the breast's lobular structure to more differentiated lobules [25]. This process of differentiation significantly reduces cell proliferation in the mammary gland [5]. Hence, the

fact of having the first child at an early age may entail lower sensitivity of differentiated mammary tissue to the action of mitogens and mutagens.

With regard to breastfeeding, the combined evidence from the Oxford Collaborative Group's reanalysis of 47 epidemiological studies indicates that lactation is consistently related to reduced breast cancer risk, decreasing by 4.3% for every 12 months of breastfeeding [26]. Nevertheless, the association between maternal lactation and MD is not clear at present. In our study, density was observed to increase with an increase in the duration of lactation in the first child, and to show a slight increase with each woman's cumulative lifetime lactation, taking age of first gestation and number of children into account in both cases. Other authors, however, report detecting no association whatsoever between lactation and density [27–30], and some studies have even described an inverse association [10, 31]. As far as we are aware, only one previous study has examined the association between cumulative lactation and high-density mammographic parenchymal patterns in a rural population in northern Greece [10], and it reported an inverse association among pre-menopausal women.

According to Russo et al. [4] the postpartum breast retains more glandular tissue than if pregnancy and lactation had never occurred, until menopause is reached and involution begins. This hypothesis could explain the greater prevalence of high mammographic density detected in the women in our study who had breastfed more recently, as well as a greater preservation of glandular tissue among those women who had breastfed for longer periods. On the other hand, prolactin is a polypeptide hormone involved in the growth and development of the mammary gland (mammatogenesis), synthesis of milk (lactogenesis), and maintenance of milk secretion (galactopoiesis) [32, 33]. As a mitogen, prolactin is also implicated in the pathogenesis and progression of human breast cancer [32]. Insofar as MD is concerned, previous studies have reported a positive association between high levels of this hormone and MD in post-menopausal women [32, 34–36].

The possible association between birth weight and the MD of adult women has been analysed by a number of studies. Among these, Cerhan et al. [16] detected a positive association, mainly in post-menopausal women. This same result was observed in a study covering 893 post-menopausal Swedish women [17]. Other studies in contrast have either detected no association [37, 38] or reported an inverse relationship [39]. To the best of our knowledge, however, no article to date has analysed the association between maternal MD and children's birth weight. The positive association detected in our study might be due to variations in the mother's hormonal levels during pregnancy. Indeed, children's elevated birth weight has been associated with high maternal levels of oestrogens and insulin-like growth factor

I (IGF-I) during pregnancy [40–42]. Although most studies which have examined the percentage of MD and circulating levels of ovarian hormones have found no and/or an inverse association, serum-IGF-I levels have nevertheless been positively associated with this phenotype, fundamentally among pre-menopausal women [9].

Hormonal factors may also be responsible for the greater prevalence of high MD detected in women who have borne twins in our study. This result has not been previously reported, though higher levels of oestrogen [40, 43–47], progesterone [45], testosterone [44], gonadotropins [40, 47–49], alpha-fetoprotein [45], and human placental lactogen [45, 47] have been described in mothers who have given birth to twins. There is the possibility that some of these women who had twins might have received previous fertility treatment, and that the greater prevalence of high MD could have been due to such treatments. In our study, however, only four women with twins had received prior treatment, so that the association between MD and the birth of identical twins would not be attributable to this type of treatment.

## Conclusions

Among parous women, our study shows a greater prevalence of high MD in mothers of advanced age at first birth, women who have borne twins, women who have breastfed for longer periods, and those whose first child had an elevated birth weight. These results might in part be accounted for by the influence of cumulative exposure to hormones and growth factors, acting as breast mitogens that could modify the composition of the stroma and mammary epithelium.

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**Conflict of interest** The authors declared that they have no conflict of interest.

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