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Maternal Breastfeeding History and Alzheimer's Disease Risk

Molly Fox[a,*], Carlo Berzuini[b] and Leslie A. Knapp[a,c]
[a]*Division of Biological Anthropology, University of Cambridge, Cambridge, UK*
[b]*Centre for Biostatistics, Institute of Population Health, University of Manchester, Manchester, UK*
[c]*Department of Anthropology, University of Utah, Salt Lake City, Utah, USA*

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Abstract. The effect of early and midlife factors on later-life cognitive function has attracted scientific and public interest in recent years, especially with respect to hormonal risk factors for dementia. There is substantial evidence for reproductive history affecting Alzheimer's disease (AD) etiology. Here, we demonstrate how breastfeeding history affects women's risk of AD. Reproductive history data was collected, and AD diagnostic interviews were performed, for a cohort of elderly British women. Using Cox proportional-hazard models, we find that longer breastfeeding duration corresponded to reduced risk of AD ($p < 0.01$, $n = 81$). Women who breastfed had lower AD risk than women who did not breastfeed ($p = 0.017$, $n = 81$). Breastfeeding practices are an important modifier of cumulative endogenous hormone exposure for mothers. Ovarian hormone deprivation and/or insulin sensitivity benefits of breastfeeding may be responsible for the observed reduction in AD risk. Future studies concerning hormone effects on AD risk should consider how reproductive history leads to variation in endogenous hormone exposure and how this may influence the relationship between hormones and AD.

Keywords: Alzheimer's disease, breastfeeding, estrogen, hormones, lactation, reproductive history, risk factors

INTRODUCTION

The health effects of breastfeeding for both mother and child are of universal relevance. Research has generally focused on health benefits for the infant, but there are also important consequences for maternal health. Some studies have explored the possibility that variation in reproductive patterns may explain some of the variation in disease risk among women (reviewed below). A range of evidence suggests that there may be a connection between the physiological effects of lactation and Alzheimer's disease (AD) etiology, warranting an investigation of the relationship between breastfeeding history and AD. Here, we explore the relationship between breastfeeding history and AD risk in a cohort of elderly British women. Previous studies established lactation's effects on maternal risk of other diseases, and previous studies have already established that aspects of reproductive history can affect AD risk.

Lactation alters disease risk

There is precedent to suggest that breastfeeding history may affect maternal disease risk. Firstly, breastfeeding history is known to alter women's breast cancer risk, including receptor-positive and triple-negative cancers, via changes in estrogen exposure and differentiation of breast tissue [1–3] (for reviews see [4, 5]). Additionally, longer breastfeeding history leads to lower rates of osteoporosis via beneficial changes in bone mineral density and bone metabolism [6]. It is possible that these benefits may be partly counterbalanced by lactation's anti-estrogenic effect, as estrogen depletion is known to increase risk of osteoporosis. But the overall effect remains that women who breastfed for longer had lower rates of osteoporosis, attributable to lactation decreasing maternal calcium metabolism [7]. Also, given prolactin's role in T-cell proliferation,

*Correspondence to: Dr. Molly Fox, Department of Biological Anthropology, University of Cambridge, Pembroke Street, Cambridge CB2 3QY, UK. Tel.: +01223 335638; E-mail: mf392@cam.ac.uk.

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it is no surprise that women with certain autoimmunities experience increased disease activity during breastfeeding. This pattern has been observed for women with Crohn's disease [8], rheumatoid arthritis, and unspecified inflammatory polyarthritis [9]. Women with multiple sclerosis exhibit the opposite pattern [10], but this has not been observed consistently [11].

Reproductive history alters AD risk

Many previous authors have suggested that women's reproductive history could affect AD risk through changes in estrogen exposure. Studies have investigated how reproductive span (years between menarche and menopause) [12–25], age at first birth [13], and parity [18, 26, 27] affect later-life cognitive function and AD risk through their effects on estrogen exposure. Previous authors have presumed that aspects of reproductive history that correspond to lower estrogen exposure should be associated with greater AD risk. While there has been support for such a trend (reproductive span [13–15, 18–21]; age at first birth [13]; fertility [18, 23, 26, 28]; parity [22]), some studies have reported the seemingly surprising result that reproductive history features indicating higher estrogen exposure increase late-life cognitive decline (e.g., age at first birth [21]; fertility [13]; parity [12, 21, 23, 27], reproductive span [25]), or no association (reproductive span [12, 23, 24]; parity [13, 14, 26]).

Additionally, estrogen replacement therapy (ERT) modifies lifetime exposure to estrogen and its effect on AD risk has been investigated, most notably in the Women's Health Initiative Memory Study (WHIMS) [29] which found no significant difference in AD incidence between ERT users and the placebo group, although there was a non-significant ERT-associated increase in incidence [30]. Other studies have found beneficial effects of ERT against AD risk, but more have found neutral or detrimental effects (for meta-analysis see [31]). Further evidence suggests that a "critical window" starting ERT within a decade of menopause is necessary to realize the estrogenic neuroprotection against AD risk [32–34].

These studies provide evidence that breastfeeding has the capacity to influence disease risk by altering hormone profiles, and reproductive history has the capacity to influence AD risk by altering hormone profiles. To date, only one previous study has reported upon the effect of breastfeeding duration on AD risk. Heys and colleagues [21, 35] found that shorter average breastfeeding duration corresponded to greater dementia risk among elderly women.

METHODS

Female patients and controls over the age of 70 and their family member(s) and/or carer(s) were recruited through nursing homes, churches, retirement community centers, the Alzheimer's Society (UK charity), and a retired employee community (Table 1) using a case-control study design. AD prevalence is positively biased in this cohort, so as to compare controls and affected individuals. Participants received a modest gift voucher for a British retail store. The protocol had approval from the University of Cambridge Human Biology Research Ethics Committee.

Each session consisted of an interview collecting information about reproductive history, determining dementia status, and collecting information about factors that would potentially confound the relationship between AD and hormone exposure. Exclusionary criteria included non-Alzheimer's-type dementia (e.g., vascular; Parkinsonian) or any possible external injury to the brain (e.g., head impact injury, brain tumor). Dementia status was measured by the Clinical Dementia Rating (CDR) scale by a researcher certified in CDR rating by the Washington University School of Medicine Alzheimer's Disease Research Center Memory & Aging Project, a certification process that requires high inter-rater reliability between the trainee and the "gold-standard" [36]. High inter-rater agreement has been demonstrated in multiple studies for clinicians and non-clinical investigators including PhDs and research assistants [37–39]. The CDR consists of a 60–90 minute interview conducted in two parts, one with the proband and the other with an informant (her relative or carer).

In the CDR, probands are evaluated in six categories: memory; orientation; judgment and problem solving; home and hobbies; community affairs; and personal care. CDR composite scores were computed: 0 = no dementia, 0.5 = questionable, 1 = mild, 2 = moderate, 3 = severe. The "sum of boxes" (SOB) was used as a continuous variable, as has become standard in clinical trials [40], computed from the sum of each category score creating a scale from 0–18. Based on published norms of disease progression [41] (typical number of years spent in each dementia phase), a new scale was created to estimate age at onset based on CDR-SOB score at time of interview (see Supplementary Data). We determined years-since-onset estimates for each possible CDR-SOB score by interpolating CDR-SOB scores between the endpoints of other scales' categories (see Supplementary Tables 1 and 2). With the aid of this scale, we estimated age at onset for each

Table 1
Participant recruitment and cohort descriptive statistics. Each row represents a different variable. Numbers in parentheses present the breakdown for probands with CDR-SOB = 0 /CDR-SOB>0. Any effect of age at participation is corrected by back-extrapolating age-at-onset. Controls are included in Cox models as right-censored cases. NA, Not available

Year of interview:	2011 : 62 (34/28)	2012 : 19 (7/12)				
Interview Location:	Participant's home: 38 (24/14)	Nursing home: 22 (4/18)	Cambridge Univ. office: 21 (13/8)			
Participant's place of birth:	Cambridge: 16 (9/7)	London: 16 (11/5)	Other Southern England: 14 (7/7)	Northern England: 12 (2/10)	Scotland, Wales, Ireland: 20 (10/10)	Outside UK: 3 (2/1)
CDR-SOB>0:	False: 41 ("controls")	True: 40 ("patients")				
Any hormone replacement therapy:	No: 59 (26/33)	Yes: 19 (15/4)	NA: 3 (0/3)			
Any breastfeeding	No: 15 (4/11)	Yes: 66 (37/29)				
Hysterectomy	No: 50 (21/29)	Yes: 23 (12/11)	NA: 8 (8/0)			
Bilateral oophorectomy	No: 63 (28/35)	Yes: 4 (2/2)	NA: 14 (11/3)			
Religion:	Church of England: 66 (35/31)	Church of Scotland/Wales: 3 (1/2)	Other Christian: 11 (5/6)	Jewish: 1 (0/1)		
Education	Schooling to age 16 or less: 71 (34/37)	Schooling beyond age 16 : 10 (7/3)				
Occupation	No work: 4 (1/3)	Factory, land, odd jobs, telephonist, technician: 14 (4/10)	Secretary, clerical, librarian, teacher, nurse: 30 (22/8)	Fashion, retail, design: 8 (6/2)	Social worker, consultant, architect: 3 (2/1)	NA: 22 (6/16)
Smoking history	Never or < 1 year: 48 (29/19)	1–10 years: 6 (4/2)	10–20 years: 6 (2/4)	>20 years: 21 (6/15)		
Alcohol consumption:	≤2 servings per day: 74 (39/35)	>2 servings per day: 7 (2/5)				

woman who at interview time had CDR > 0, defined as when CDR-SOB would have gone from 0 to 0.5, back-extrapolated from the observed degree of dementia at time of interview.

Accuracy of recall data

Information in the present study on probands' breastfeeding history came directly from the probands, and was often confirmed or independently remembered by probands' spouses and/or children. Husbands ($n = 10$) in particular were useful in breastfeeding duration recall. A study measuring elderly women's recall of breastfeeding, measured 34–50 years after last breastfeeding occurred, found that women were able to recall whether or not they had breastfed with 94% accuracy for each of their children [42]. As for breastfeeding duration, the study found that under- and over-reporting occurred with equal frequency, and 55% of women were able to recall breastfeeding duration per child accurately within 1 month and 71% within 2 months [42].

Variables

"AD-free time" was defined as the retrospectively estimated number of years in excess of age 50, prior to the interview, during which the woman was free from AD. The AD-free time for those women who were judged to be free from AD was treated as right-censored at the time of the interview, as is common practice in survival analysis. The dependency of AD-free time on the predictive variables was analyzed via Cox proportional hazards model.

In a main-effects analysis, each continuous predictive variable contributes into Cox model a coefficient, "coef," whose value is estimated on the basis of the data. When exponentiated, exp(coef), this parameter yields the ratio of the hazards (probability of AD onset per unit time) between two hypothetical women who are identical except for a unit difference between their respective values of the predictive variable (corresponding to an exp(1) ratio of the two values when the variable has been log-transformed).

This analysis included only those women who had at least one child, in order to study the effects of lactation without the confounding differences between nulliparous and parous women. Our analysis focused on the effect of breastfeeding, as measured via (1) BFSUM: the total sum of months spent breastfeeding; (2) BFMEAN: the mean breastfeeding per full-term pregnancy; (3) BFSUM/PMONTHS: the ratio between total sum of months spent breastfeeding and total sum of months spent pregnant; and (4) BFANY: a binary variable expressing whether there was any breastfeeding, upon hazard of AD. The reason we considered the ratio variable (3) is that it included the total number of months spent pregnant which takes into account miscarriages and abortions. If there is a biological effect of pregnancy to consider, then this effect may exert regardless of whether the pregnancy was full-length, and for some women, inclusion of lost pregnancies made for a substantially more accurate reflection of reproductive history. The effects of lactation that could affect AD risk (ovarian hormone suppression, restoration of glucose metabolism, upregulated inflammation) are all opposite to the effects of pregnancy, and so we chose to consider not only breastfeeding history in itself, but also months spent breastfeeding compared to months spent pregnant in lifetime.

Statistical methods

Prior to analysis, continuous predictive variables with a strongly skewed distribution were transformed by adding a constant and taking the logarithm, in such a way to improve the symmetry of their distribution, thus preventing the analysis results from being unduly influenced by individuals with extreme values of a predictive variable.

We suspected that our predictive variables might have different effects in women based on family history. AD pathogenesis among those individuals who carry pro-inflammatory alleles may be different than among those AD patients who do not, as the genetic contribution to late-onset AD risk appears to be pro-inflammatory alleles, and carrying even a small number of pro-inflammatory alleles may increase AD risk 10-fold [43]. We felt there was a chance that the impact of the predictive variables on hazard of AD may have different magnitude in women with and without AD family history.

The above considerations motivate the assessment of homogeneity of the effect across two groups of women: those with and without dementia family history. We defined "family history of dementia" as having a parent or sibling who likely had dementia, as reported by the proband and/or family members. This was the most accurate information possible, as AD was seldom officially diagnosed in early 20th century Great Britain, and this is when the probands' parents would have been elderly. We checked for a possible statistical interaction between the AD-inducing effect of each predictive variable and family history, via partial likelihood com-

parison of a Cox model that contains the relevant term of interaction with the corresponding main-effects model. Whenever the interaction turned out to be significant, we decided that we would carry out separate analyses of the prognostic import of the predictive variable in the two groups of women by family history.

The joint effect of predictive variables on hazard of AD was studied by partial likelihood test comparison of Cox models containing different subsets of the breastfeeding variables (BFSUM, BFMEAN, BFSUM/PMONTHS, BFANY). AD risk was measured as the time between age 50 and a transition from CDR-SOB = 0 to 0.5 occurring, until age-at-interview. For each predictive variable, we ran two models. The first model controlled for age-at-interview, and the second model controlled for this plus education, occupation, ERT use, oophorectomy, age at first birth, and age at menopause. Comparison of Kaplan-Meier survival curves for AD-free time was used to illustrate the main results. All analyses were performed using the R language and environment for statistical computing and graphics.

Consideration of the martingale residuals [44] is useful for discerning the legitimacy of the Cox models fitted, and in particular, for checking that analysis results have not been excessively influenced by a small number of individual cases.

RESULTS

To investigate the role of breastfeeding history on AD risk in a cohort of British women, we focused on data from 81 women from our initial dataset of 133 (Table 2). All women were White British currently residing in England. Ten cases were excluded from analysis due to factors that would cause non-Alzheimer's-type dementia (e.g., stroke) or unusual hormone exposure (e.g., ovarian cancer). Nulliparas ($n = 13$) were excluded from these statistical analyses, and cases with missing information on breastfeeding history ($n = 9$) or missing information on family history of dementia ($n = 20$) were also excluded (Table 2). The preliminary analysis included 81 cases: 41 women with CDR-SOB = 0 ("controls"), and 40 women with CDR-SOB>0 ("patients"). When the relevant interaction term turned out to be significant, an additional analysis was performed that focused on the subset of women with no family history of dementia: 33 women with no sign of dementia and 28 women with any sign of Alzheimer's-type dementia. For patients ($n = 40$), the mean age at estimated onset (shift from CDR-SOB = 0 to 0.5) was 74.79 years of age. See Tables 1 and 3 for cohort demographics and reproductive history statistics.

The variables we considered were: (1) BFSUM, (2) BFMEAN, (3) BFSUM/PMONTHS, and (4) BFANY. To enhance the symmetry of the distribution of the predictive variables, we added 0.1 to the values of BFSUM, and then took logarithms. An analogous transformation was applied to BFMEAN, this time by adding the constant 0.2. A transformed version of the BFSUM/PMONTHS variable, defined by log((BFSUM+0.1)/(PMONTHS+5)), was used in the analysis. After these transformations, variables BFMEAN and BFSUM/PMONTHS were found to be extremely correlated with each other. Consequently, we decided to exclude BFMEAN from subsequent analysis.

An interaction between family history of dementia and each of BFSUM and BFSUM/PMONTHS (but not BFANY) was found on the basis of our data ($p = 0.05$). Subsequent analysis showed no evidence of an AD-inducing effect of any of the breastfeeding variables within women with family history of dementia, as the relevant coefficient estimates had confidence intervals centered around the null value. In contrast, significant effects for the predictive variables on hazard of AD were found from an analysis of the subsample of women without family history of dementia. None of the following variables contributed significantly to the prediction of AD once the value of the breastfeeding variable is taken into account: age-at-interview, exponentiated age-at-interview, education history, age at first birth, age at menopause, ERT use, or bilateral oophorectomy. Religion, smoking history, and drinking history had no statistical effects on AD risk in this cohort. Occupation history had a statistically significant ($p < 0.05$) contribution to AD prediction in some of the models after breastfeeding variables were considered, and its inclusion in the models controls for these effects. It should be noted that occupation history was "unknown" in a disproportionate number of patients compared to controls (Table 1), potentially contributing to the strength of its statistical relationship with AD hazard.

Lifetime sum of months spent breastfeeding (BFSUM)

We found that longer breastfeeding history was significantly associated with lower AD risk in our cohort. The results from our Cox model analysis of the dependence of AD risk on BFSUM are summarized in

Table 2
Missing values. This table reports the number of cases for which we were able to collect essential information for this study. Statistics were initially performed on the resulting cohort of 81, and when excluding probands with family history of dementia (an additional 20 cases), those statistical models included 61 cases

	Total participants	Age at AD onset	Birthed at least one child	Breastfeeding duration history	Family history of dementia
Cases included	133	123	110	101	81
Missing values	0	10	0	9	20

Table 4. Under the assumptions of Cox model, the estimated ratio of the hazards of AD of two hypothetical women who are identical except that one of them has an exp(1)-fold higher value of BFSUM, is 0.78, the lower hazard being for the woman with higher BFSUM. In other words, a woman who breastfed cumulatively for 2.72-times as many months as another woman would have a 22% reduction in AD risk if they were identical besides their difference in breastfeeding history. For example, comparing two hypothetical women who are identical except one breastfed cumulatively for 12 months and the other 4.41 months, the one who breastfed for 12 months would have only 78% of the AD risk than the other woman has. A plot of the martingale residuals revealed that the model fit was not unduly influenced by a small number of cases.

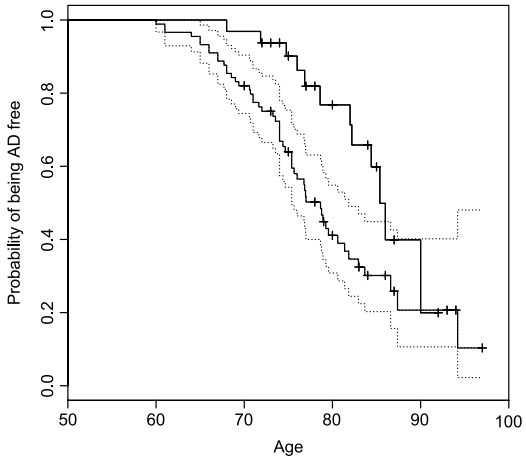

Fig. 1. Women with higher breastfeeding-to-pregnancy ratio have lower AD risk. For each value of age, the plot reports the probability of being event-free for women with BFSUM/PMONTHS lower than the sample median (lower curve) and for women with BFSUM/PMONTHS above the sample median (upper curve). Pointwise 95% confidence bands for the lower curve are also shown (dotted lines). Age at event refers to estimated age at shift from CDR-SOB = 0 to CDR-SOB > 0. This plot gives a visual sense of the magnitude of the effect. The Cox model reported in Table 5 represents a more meaningful analysis utilizing the detailed information available for BFSUM/PMONTHS.

Number of months spent breastfeeding divided by number of months spent pregnant in lifetime (BFSUM/PMONTHS)

This variable measured the total number of months a woman spent breastfeeding and the total number of months a woman spent pregnant, including miscarriages, abortions, stillbirths, and childbearing. We found that a higher breastfeeding-to-pregnancy ratio corresponded to significantly lower risk of AD (Fig. 1). A plot of the martingale residuals revealed that the model fit was not unduly influenced by a small number of cases. The results from our Cox model analysis of the dependence of AD risk on BFSUM/PMONTHS are summarized in Table 5. Under the assumptions of the Cox model, the estimated ratio of the hazards of AD of two hypothetical women who are identical except that one of them has an exp(1)-fold higher value of BFSUM/ PMONTHS, is 0.77 (Model 1) or 0.76-0.79 (Model 2), the lower hazard being for the woman with higher BFSUM/PMONTHS. For example, comparing two hypothetical women who are identical except one breastfed for 8 months and was pregnant for 8.8 months, and the other woman breastfed for 6 months and was pregnant for 18 months, the former woman would have only 77% (or 76–79%) of the AD risk that the latter woman has. For a further example, comparing two hypothetical women who are identical and breastfed cumulatively for the same amount of time, but one was pregnant for 9 months and the other was pregnant for 24.5 months, the former woman would have only 77% (or 76–79%) of the AD risk that the latter woman has.

Any breastfeeding (BFANY)

Women who breastfed exhibited reduced AD risk compared to women who did not breastfeed at all (Fig. 2). The results from our Cox model analysis of the dependence of AD risk on BFANY are summarized in Table 6. Under the assumptions of Cox model, the estimated ratio of the hazards of AD for two hypothetical women who are identical except that one of them breastfed is 0.36 (or Model 2, 0.30), the lower hazard

Table 3
Cohort age and reproductive features. Description of subset of cohort included in all analyses. Subset defined as parous probands who have complete CDR, breastfeeding, and family history information. For "Controls" (CDR-SOB = 0) column, $N = 41$. For "Patients" (CDR-SOB>0) column, $n = 40$

	Controls				Patients			
	Median	Min	Max	SD	Median	Min	Max	SD
Age at interview	80	72	97	6.36	86	72	98	5.80
Age at menopause (years)	50	36	60	5.29	50	33	60	5.72
Duration ERT (months)	0	0	228	60.06	0	0	120	19.70
Number of full-term pregnancies	2	1	5	1.07	2	1	10	1.68
Age at first birth	26	17	40	4.62	25	20	39	4.25
Mean breastfeeding duration per full-term pregnancy (months)	5	0	12	3.12	2.36	0	12	3.55
Cumulative breastfeeding duration (months)	11	0	48	11.54	6	0	51	11.26

Table 4
Longer breastfeeding history is associated with lower AD risk. Cox model analysis of the dependence of AD risk on BFSUM. BFSUM is the cumulative number of months spent breastfeeding in lifetime. The parameter used was log(BFSUM + 0.2). $*p < 0.05$; $**p < 0.01$. "Fam hist" refers to whether individuals had a parent or sibling with dementia. Model 1: Controlling for age at interview and exponentiated age. Model 2: Controlling for age at interview, exponentiated age, education, ERT use, bilateral oophorectomy, age at first birth, and age at menopause. The table reports the partial likelihood point estimate for the effect of the log-transformed BFSUM variable, the corresponding exponentiated value, the standard error, the p-value for the relative sharp null hypothesis and the 95% confidence interval. The partial likelihood ratio test p-value for the null hypothesis of no effect was 0.042 (Model 1 all cases), 0.024 (Model 1 no fam hist), 0.010 (Model 2 all cases), and 0.013 (Model 2 no fam hist). The score logrank test p-value was 0.032 (Model 1 all cases), 0.014 (Model 1 no fam hist), 0.012 (Model 2 all cases), and 0.008 (Model 2 no fam hist). There were 40 observed failure events in models with all cases, 28 observed failure events in models including only cases with no fam hist, and 12 in models including only cases with fam hist. In Model 2, probands' occupation was flagged as significant $p < 0.05$). No other control variable made a statistically significant contribution to the model fittings. The two models fitted on the basis of the women with fam hist ($n = 20$) showed no evidence of an effect on AD risk in this subset (Model 1: $p > 0.05$; Model 2: monotone likelihood)

Model	n	coef	exp(coef)	se(coef)	p-value	96% CI
1	81 (all cases)	−0.25	0.78	0.09	0.0035**	(0.66, 0.92)
1	61 (no fam hist)	−0.25	0.78	0.09	0.0038**	(0.66, 0.92)
1	20 (fam hist)	0.09	1.09	0.16	0.587	(0.80, 1.48)
2	81 (all cases)	−0.26	0.77	0.09	0.0079**	(0.64, 0.93)
2	61 (no fam hist)	−0.23	0.79	0.09	0.0198*	(0.65, 0.96)
2	20 (fam hist)	n/a	n/a	n/a	n/a	n/a

Table 5
Higher breastfeeding-to-pregnancy ratio is associated with lower AD risk. Cox model analysis of the dependence of AD risk on BFSUM/PMONTHS. BFSUM and PMONTHS are the cumulative number of months spent breastfeeding and pregnant in lifetime. The parameter used was log((BFSUM+0.1)/(PMONTHS+5)). $*p < 0.05$; $**p < 0.01$. "Fam hist" refers to whether individuals had a parent or sibling with dementia. See Table 1 caption for description of Models 1 and 2. This table reports the partial likelihood point estimate for the effect of the log-transformed BFSUM/PMONTHS variable, the corresponding exponentiated value, the standard error, the p-value for the relative sharp null hypothesis and the 95% confidence interval. The partial likelihood ratio test p-value for the null hypothesis of no effect was 0.033 (Model 1 all cases) 0.017, (Model 1 no fam hist), 0.009 (Model 2 all cases), and 0.013 (Model 2 no fam hist). The score logrank test p-value was 0.022 (Model 1 all cases), 0.008 (Model 1 no fam hist), 0.009 (Model 2 all cases), and 0.006 (Model 2 no fam hist). There were a total of 40 observed failure events in models with all cases, 28 observed failure events in models including only cases with no fam hist, and 12 in models including only women with fam hist. In Model 2, probands' occupation was flagged as significant $p < 0.05$). No other control variable made a statistically significant contribution to the model fittings. The two models fitted on the basis of the women with fam hist ($n = 20$) showed no evidence of an effect on AD risk in this subset (Model 1: $p > 0.05$; Model 2: monotone likelihood)

Model	n	coef	exp(coef)	se(coef)	p-value	96% CI
1	81 (all cases)	−0.26	0.77	0.09	0.0022**	(0.65, 0.91)
1	61 (no fam hist)	−0.27	0.77	0.09	0.0022**	(0.65, 0.91)
1	20 (fam hist)	0.11	1.12	0.17	0.53	(0.79, 1.57)
2	81 (all cases)	−0.27	0.76	0.09	0.0057**	(0.63, 0.92)
2	61 (no fam hist)	−0.23	0.79	0.09	0.0197*	(0.65, 0.96)
2	20 (fam hist)	n/a	n/a	n/a	n/a	n/a

being for the woman who breastfed. In other words, a woman who breastfed would have a 64% reduction in AD risk if they were identical besides their difference in breastfeeding history. A plot of the martingale residuals revealed that the model fit was not unduly influenced by a small number of cases.

Table 6
Women who breastfed had lower AD risk. Cox model analysis of the dependence of AD risk on BFANY. BFANY is a binary variable representing whether parous women breastfed any of their children for at least one week. *$p < 0.05$; **$p < 0.01$. See Table 1 caption for description of Models 1 and 2. This table reports the partial likelihood point estimate for the effect of the BFANY variable, the corresponding exponentiated value, the standard error, the p-value for the relative sharp null hypothesis and the 95% confidence interval. The partial likelihood ratio test p-value for the null hypothesis of no effect was 0.12 (Model 1) and 0.14 (Model 2). The score logrank test p-value was 0.10 (Model 1) and 0.11 (Model 2). Both models were fitted on the basis of 81 sample individuals for a total of 40 observed failure events. In Model 2, probands' occupation was flagged as significant ($p < 0.05$). No other control variable made a statistically significant contribution to the model fittings

Model	n	coef	exp(coef)	se(coef)	p-value	96% CI
1	81 (all cases)	−1.01	0.36	0.42	0.0169*	(0.16, 0.83)
2	81 (all cases)	−1.21	0.30	0.46	0.0089**	(0.12, 0.74)

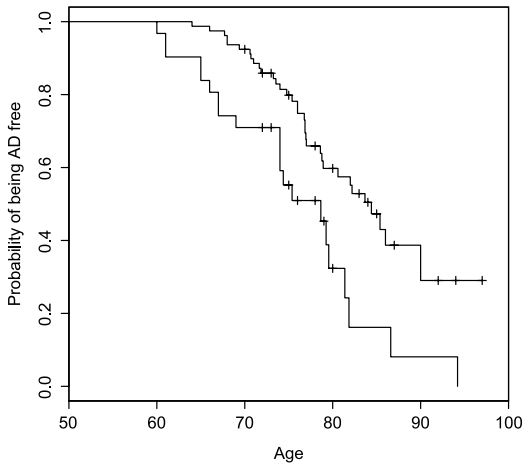

Fig. 2. Parous women who breastfed have lower AD risk. For each value of age, the plot reports the probability of being event-free for parous women who did not breastfeed (lower curve) and who did breastfeed (upper curve) (see Table 5). Age at event refers to estimated age at shift from CDR-SOB = 0 to CDR-SOB>0.

DISCUSSION

In a cohort of British women, longer breastfeeding duration was associated with diminished AD risk. Factors that could account for the observed relationship between breastfeeding history and AD risk include both sociological and physiological explanations. Different levels of education or socio-economic factors could correlate with breastfeeding rates, and could thereby account for our findings. To this end, we controlled for education, which ultimately did not contribute to the models' significance, and for occupation, which did contribute significantly to some of the model fittings. We did not collect direct socio-economic status (SES) information for our cohort. Some studies have found AD risk to be negatively correlated with SES, while others including the WHIMS have found no such effect [45]. Karp and colleagues found that the statistical effect of SES disappeared when educational attainment was entered into the model [46]. The significant impact of educational attainment (or perhaps another variable for which education is merely a proxy, such as nutrition) eliminated the statistical effect of SES on AD risk [46].

The significantly protective effect of high occupational status in this cohort is consistent with the findings of some other studies, but not all, which observed negative correlations between occupational status and AD risk (see [47] for meta-analysis). Controlling for the effects of education and occupational status in our models, we still found a highly significant impact of breastfeeding upon hazard of AD, suggesting that the effect of breastfeeding on AD risk can be explained by factors other than these. By controlling for both education history and occupation history in our analyses, SES issues were at least partially addressed. Potential physiological explanations are described below.

Ovarian hormone exposure

The negative sign of the estimated effect of breastfeeding on AD risk, corresponding to a protective effect, may appear to be in contrast with estrogen's well-documented protective effect against AD, because during periods of intensive lactation, estrogen (and progesterone) levels are typically negligible [48, 49]. As breastfeeding becomes less frequent and duration of feeding shorter, the anovulatory effects of lactation decrease, and menstrual cycling may resume [48]. Breastfeeding duration can be considered a proxy for the amount of time spent in a state of estrogen and progesterone deprivation after each pregnancy. It should be noted that both estrogen and progesterone have been observed to have both beneficial and deleterious effects on cognitive health in general, and AD risk in particular. Potentially, duration of breastfeeding-associated estrogen and/or progesterone deprivation confers protection against AD. It may seem less likely that estrogen-deprivation is advantageous,

as estrogen protects the brain against AD-specific brain insults including amyloid cascade [50], oxidative stress [51], inflammation [52], tau hyperphosphorylation [53], neuronal apoptosis [54], and acetylcholine suppression [55], among other neuroprotective functions. Nevertheless, some studies have found evidence of estrogen-associated deleterious effects on late-life cognitive function and AD risk [12–14, 21, 23–27, 29, 30]. Progesterone also has a heterogeneous effect on the brain, specifically in relation to AD. Progesterone exposure may desensitize the brain estrogen receptors [56], attenuating estrogen's ability to protect the brain against the AD neuropathogenesis. Rodent studies have demonstrated that progesterone administration blocks estrogen's beneficial effects on brain function and cognitive performance [57], attenuates estrogen-induced neuroprotection [58], and while estrogen administration inhibited the AD-like cascade and cognitive impairment in an AD mouse model, co-administration with progesterone restored AD neuropathy and cognitive impairment [59].

Longer breastfeeding duration could be considered a proxy for reduced ovarian hormone exposure. Our results indicate a potentially more complex picture than the current literature suggests, and exposure to estrogen and/or progesterone may have beneficial or deleterious effects on risk of AD in different contexts. For example, one possible explanation for our results could be a benefit from progesterone deprivation. Potentially, progesterone deprivation experienced during lactation may be a natural compensation for any deleterious effects from the high levels of progesterone during pregnancy. Women who experienced phases of high progesterone (i.e., pregnancy) without compensatory phases of nearly no progesterone (i.e., breastfeeding) may be more vulnerable to AD due to estrogen receptor insensitivity in the brain. Further research is necessary to elucidate how breastfeeding's effect on ovarian hormones might modify AD risk.

Insulin sensitivity

Another possible factor in breastfeeding's protection against AD onset might be its role in restoring glucose tolerance. Pregnancy induces a natural state of insulin resistance throughout the periphery [60, 61] and breastfeeding restores insulin sensitivity [62]. This is evident in skeletal muscle and adipose tissue [63], with maternal insulin-mediated glucose disposal decreasing by approximately 50% [64]. Higher parity appears to increase risk of type-2 diabetes mellitus [65]. Lactation is known to reverse this natural state of insulin resistance and women who breastfeed experience improved insulin sensitivity [66]. Longer breastfeeding history is also associated with lower risk of type-2 diabetes decades later in life [67]. The low levels of estradiol during lactation may be responsible for the beneficial effect of lactation on maternal glucose tolerance [68].

The role of insulin insensitivity in AD pathology is so important that AD is sometimes referred to as "type-3 diabetes" [69]. The malfunction of glucose utilization and energy metabolism in the brain may be a proximate mechanistic explanation for much of AD neuropathology [69, 70]. In mouse models of AD, treatment with insulin-sensitivity increasing drugs reduced learning and memory deficits [71], and in humans with AD, insulin administered intra-nasally improved cognitive performance [72], and reduced plasma amyloid-β [73].

Our finding that more months spent breastfeeding may protect against AD (Table 4) would be consistent with the fact that breastfeeding has a beneficial effect on insulin sensitivity, and insulin sensitivity may be protective against AD development. Women who spent more time pregnant without a compensatory phase of breastfeeding may have more impaired glucose tolerance, which is consistent with our observation that women who spent more months pregnant compared to breastfeeding had increased risk of AD (Fig. 1, Table 5).

Family history of dementia

For women without a parent or sibling with dementia ($n = 61$), breastfeeding decreased risk. For women with a parent or sibling who had dementia ($n = 20$), the impact of breastfeeding on AD was significantly lower than for women without family history of dementia and not significantly different from no effect (Tables 4 and 5). While we do not have enough statistical power to say anything about the role of breastfeeding within the group of women with family history of dementia ($n = 20$), we do have enough data to confirm that breastfeeding has a different role between the two groups of women with and without such family history. Because the interaction is significant, it is no longer justified to analyze the two groups together, and so we present separate analyses for the two groups (Tables 4–6). We cannot confirm why the effect of breastfeeding on AD risk is so different between these two groups of women, but potential explanations deserve further investigation.

Our interaction means that we have found that the role of breastfeeding changes according to family history of dementia. Related findings have been observed

in two previous studies. One study found that the effect of reproductive span on AD risk changes according to whether or not a woman carried an ApoE-ε4 allele. Among ε4 carriers, longer span was associated with elevated AD risk [25]. Another study found that only among women who did not carry an ε4 allele, nulliparas had later AD onset, and no such effect was observed for women who carried an ε4 allele [28]. Although we cannot be sure, our observed interaction with family history of dementia could be a consequence of the same phenomenon observed by these authors. Our study design did not include genotyping, so we can only postulate that women with family history of dementia were more likely to carry pro-inflammatory alleles [43], accounting for the similar findings from previous studies.

Given the possibility that prolactin's pro-inflammatory effects are limited to already upregulated immunological conditions [74], this could explain why the beneficial effect of breastfeeding was significantly reduced in women with family history of dementia. Evidence from a range of sources [75–77] indicates that prolactin exacerbates inflammation, but prolactin does not seem to induce inflammation in basal conditions [78–80]. Although increased prolactin exacerbates disease activity in patients with autoimmunity, greater or longer exposure to prolactin does not increase risk of autoimmunity in healthy women [81]. Future research should explore whether breastfeeding promotes AD risk in women with pro-inflammatory alleles through increased prolactin levels.

Study strengths and limitations

Gathering breastfeeding history data was a central aim of the study design. Total duration of pregnancies during an individual's lifetime was calculated to include miscarriages and abortions, a novel and highly informative approach. Rather than relying on only the proband for breastfeeding history and other aspects of reproductive history, the interview process included other family members and written sources.

The age at which women might have transitioned from CDR-SOB = 0 to 0.5 was estimated based on current degree of dementia. This method is imperfect, although there is no reason to suspect that the directionality of error particularly favors earlier or later onset, and no indication that bias in this methodology would be statistically related to breastfeeding history. This method was chosen, rather than clinical diagnosis or family or proband reported age at onset, because the early signs of AD are often purposefully concealed by patients, ignored, exaggerated, misunderstood, or unrecognized by family members. Clinical diagnosis could occur at any stage of disease progression, and different clinicians would vary in their timing and threshold for diagnosis. However, this method is merely a rough estimation and disease progression timing varies between patients. We hope our results demonstrate justification for longitudinal study of this topic.

Subjects and/or their families volunteered directly, but no possible biases in our cohort were correlated with predictive variables, as participation and refusal were not related to breastfeeding history, and recruitment material advertised without mentioning reproductive history. This study only considered White British women, and future research should explore other ethnic and regional groups.

CONCLUSIONS

This study considers how women's breastfeeding history affects AD risk. We utilized information collected from interviews of 133 British women, ultimately focusing our analysis on 81 cases with sufficient information. We estimated age at transition from CDR-SOB = 0 to 0.5 among women with CDR-SOB scores above 0 as a proxy for AD symptom onset. Longer breastfeeding duration corresponded to lower AD risk, and any breastfeeding versus no breastfeeding was associated with reduced AD risk. We suggest that this could be due to breastfeeding's beneficial effects on estrogen and/or insulin sensitivity. Future research should investigate the link between breastfeeding, insulin sensitivity, and type-2 diabetes. Future studies should include information on gestational diabetes, pregnancy's effect on glucose tolerance, and the link between estrogen and insulin postpartum.

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SUPPLEMENTARY MATERIAL

Supplementary methods, tables and figure are available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-130152.

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