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Author/s:

Dall, GV;Britt, KL

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Estrogen Effects on the Mammary Gland in Early and Late Life and Breast Cancer Risk

Genevieve Victoria Dall¹ and Kara Louise Britt^{1,2*}

¹Peter MacCallum Cancer Centre, Melbourne, VIC, Australia, ²The Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, VIC, Australia

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*Correspondence:

Kara Louise Britt
kara.britt@petermac.org

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A woman has an increased risk of breast cancer if her lifelong estrogen exposure is increased due to an early menarche, a late menopause, and/or an absence of child-bearing. For decades, it was presumed that the number of years of exposure drove the increased risk, however, recent epidemiological data have shown that early life exposure (young menarche) has a more significant effect on cancer risk than late menopause. Thus, rather than the overall exposure it seems that the timing of hormone exposure plays a major role in defining breast cancer risk. In support of this, it is also known that aberrant hormonal exposure prior to puberty can also increase breast cancer risk, yet the elevated estrogen levels during pregnancy decrease breast cancer risk. This suggests that the effects of estrogen on the mammary gland/breast are age-dependent. In this review article, we will discuss the existing epidemiological data linking hormone exposure and estrogen receptor-positive breast cancer risk including menarche, menopause, parity, and aberrant environmental hormone exposure. We will discuss the predominantly rodent generated experimental data that confirm the association with hormone exposure and breast cancer risk, confirming its use as a model system. We will review the work that has been done attempting to define the direct effects of estrogen on the breast, which are beginning to reveal the mechanism of increased cancer risk. We will then conclude with our views on the most pertinent questions to be addressed experimentally in order to explore the relationship between age, estrogen exposure, and breast cancer risk.

Keywords: parity, breast cancer risk, menarche, menopause, estrogens

Breast cancer remains one of the most prevalent diseases in the western world, with one in eight women predicted to be affected by breast cancer in their lifetime. Improvements in detection, anti-estrogen therapies, and cytotoxic chemotherapy have led to increased survival rates, from 72% in 1980s to 89% in 2010. Despite this, the incidence of breast cancer has increased over the same period (1, 2). This year in Australia, 15,930 women are predicted to be diagnosed with breast cancer and this figure is expected to rise to 17,210 women by 2020. Similar increases in incidence have been reported in America, the United Kingdom, and China (3–5). Estrogen receptor (ER)-positive breast cancers are the only subtype that are increasing (6–9) and as they make up 75–80% of all breast cancer cases this may explain the increase in breast cancer incidence overall. It has been postulated that the rising incidence of ER+ breast cancer is driven by hormonal risk factors such as low and/or late parity, early menarche, late menopause, as well as the use of combined oral contraceptive (OCP) pill and postmenopausal hormone replacement therapy (HRT) (10–13) rather than determinants such as BMI and BRCA1/2 status. In this review we will discuss the epidemiological data and experimental

models used to investigate the effects of these different hormonal factors on ER+ breast cancer risk.

PARITY

Childbearing and a lack thereof have been known to influence breast cancer risk ever since Bernardino Ramazzini documented an increase in breast cancer incidence in nuns in the eighteenth century (14). Over the last century, these observations have been supported by numerous epidemiological studies (15–17). Depending on the age at which childbearing begins, after a transient increase risk period immediately following pregnancy (18), parity provides lifelong protection against breast cancer by up to 50% (19). In the last two decades, large case-control and meta-analysis studies have shown that the protection provided by parity is restricted to hormone receptor positive tumors (ER+PR+) (20–22). Recent studies have attempted to investigate whether parity reduces the risk of different molecular subtypes of breast cancer but have shown conflicting results (23–25) highlighting the need for larger studies to shed light on these inconsistencies.

The older a mother is at the age of the first full-term birth, the less the protection that is instilled by the pregnancy (18, 20, 26–28). After 35 years of age, parity paradoxically increases risk compared to women who have not had children. An increasing number of births also confer protection (29–33) with additional births providing an extra 10% reduction in risk (34). In addition, the spacing between births can influence breast cancer risk, with less than 1 year or greater than 3 years providing more protection than a birth space of 1–2 years (35). The protective effect of childbearing is important to consider in relation to current reproductive trends. Recent reports have identified that more women in western cultures are remaining childless or delaying childbearing until after 35 years of age (1, 2, 36). In 2012, up to 20% of Australian women were childless, and of those that were bearing children, 24% had their first child after 35 years of age (37). It is, therefore, proposed that this decline in childbearing and increasing age at first full-term birth may be contributing to the rise in breast cancer incidence.

Considering the influence of age at first birth, number of births, and birth spacing on breast cancer risk, it is not surprising that breastfeeding is also able to modulate risk (38, 39). The reduction in breast cancer risk offered by breastfeeding is 4.3–4.5% for every 12 months of breast feeding (40, 41), a reduction that is in addition to the reduced risk following each birth. Despite these findings and a recommendation from the National Health and Medical Research Council to breastfeed for at least 6 months (42), only 50% of Australian babies were being breastfed at 4 months and this dropped to 29.7% at 9–12 months of age (43). In contrast to the protective effects of childbearing, the protection conferred by breastfeeding is not limited to ER+ breast cancer (20, 44, 45). The mechanisms of breastfeeding-induced protection are largely unknown, as are the reasons why its effects are not restricted to ER+ cancers.

In addition to the epidemiological studies in women, parity-induced protection against breast cancer has also been shown experimentally through the use of rodent models. Rodents have been used due to the similarities in morphological structure

between the mammary gland and human breast and the conservation of genes and pathways between rodent and human mammary epithelial cell subpopulations (46). Parity in rodents reduces the incidence of carcinogen-induced mammary tumors (47, 48) and, as with women, shows a dependence on age, with younger mothers showing a greater reduction in tumor incidence (49, 50). The rodent models have also shown that the protective effects of pregnancy can be simulated through the administration of pregnancy levels of estrogen and progesterone to rodents (51, 52). This provides supporting evidence that parity-induced protection may be hormonally driven, and thus may explain why its protection is restricted to ER+ breast cancers. The mechanisms underlying parity-induced protection remain an active area of research with investigators assessing the role of the mammary stem cells, ER+ cells and other growth factors (53, 54).

MENOPAUSE

Menopause is defined as the final menstrual period. It occurs when there has been a change in a woman's reproductive hormones and the ovaries no longer release any eggs. Menopause itself is not a breast cancer risk factor, but over 70% of all breast cancer diagnoses are made in women who are 50 or older, and thus postmenopausal women have a higher risk than premenopausal women. The timing of menopause has been shown to significantly affect breast cancer risk. While not documented historically, the duration of reproductive years was identified as a breast cancer risk factor in early epidemiological studies (15, 55). Large-scale case-control studies and meta-analyses have now consistently shown that younger age at menopause decreases ER+ breast cancer risk (10, 56–59), with each year older at menopause increasing the risk by 2.9–4% (10, 59).

The age at which a woman undergoes menopause varies considerably between and within ethnicities (60); however, mother-daughter and twin studies have found that only 44–63% can be accounted by heritability (61–63). Mother and daughter studies have postulated that the heritability may be driven by genetic changes in hormone expression as the maternal age at menopause was found to be a strong predictor for high follicle-stimulating hormone (FSH) levels (an indicator of ovarian aging) in daughters (64). Genetic studies have tried to ascertain what may be mediating the timing and found that polymorphisms within the ER gene and ER signaling pathway are significantly associated with age at natural menopause (65, 66). It is not clear whether these polymorphisms are associated with increased or decreased ER signaling. Larger-scale genome-wide sequencing studies (between ~3,000 and 40,000 women included compared to ~200–900 in previous studies) have confirmed this association and have also reported further polymorphisms in DNA damage and repair genes and genes associated with mitochondrial DNA, FSH, and immune components (67–70). Together, these studies only explain ~4% of the variation in age at menopause. Furthermore, only one single nucleotide polymorphism (rs2517388) for age at menopause was associated with breast cancer risk (71). The rs2517388 polymorphism is located within a gene that encodes for a subunit of the MLL histone methyltransferase protein (72). MLL has been shown to act as a coregulator

of ER-induced progesterone receptor gene activation (73). It is also involved in estrogen-dependent activation of kinesins (74), which have been linked to tamoxifen resistance. This may indirectly explain the link between the polymorphism, age at menopause, and breast cancer; however, this has not been tested functionally. Thus, the underlying reason why age at menopause affects breast cancer risk is still unknown. Women with a later menopause have been shown to have longer mean menstrual cycle length (75) than those with an average age menopause. It is not known what mediates this, but it is intriguing to think that it may be related to hormone levels in the follicular phase of the menstrual cycle seeing as the follicular phase length drives total cycle length.

Mouse models exploring the effects of menopausal age on breast cancer risk are challenging as mice do not undergo natural menopause. This may explain the lack in experimental data exploring or even confirming the abovementioned polymorphisms associated with age at menopause in animal models. However, like humans, menopause can be induced in mice by surgical removal of the ovaries. Using such a system one group have assessed the effect of timing of HRT on the postmenopausal mammary gland in an effort to explain the increase in breast cancer risk observed following HRT (76). They found that the postmenopausal gland (5 weeks post ovariectomy) was more responsive to estrogen-driven proliferation compared to their model of peri-menopause (immediately following ovariectomy). Similar studies could be performed to shed light on the underlying mechanism of an early versus late menopause as being protective against breast cancer incidence.

MENARCHE

Age at menarche, like menopause, is also associated with breast cancer risk. However, unlike age at menopause, the older a woman is at age at menarche, the lower her risk of breast cancer. Several groups have now shown that starting menses prior to 11 years of age increases the risk of breast cancer, while a later age at menarche (14 years) reduces the risk (10, 58, 59, 77–81). Sisti and colleagues showed that the relative risk of breast cancer was increased by 5% for each year younger at menarche (78) and the Collaborative group on hormonal factors reported up to an 18% reduction in risk in those girls experiencing a late menarche (≥ 13), compared to those who began cycling at 11 (10).

During the last decade, epidemiological studies reporting on trends in age at menarche have shown that irrespective of ethnicity, the average age of menarche is ~12 years (10, 82–84). Historically, age at menarche was much older (85). A review published in 1982 assessing reports on age at menarche including 220,037 European women from 1795 to 1981 observed a 2–3 month decline in age at menarche per decade (86) with some reports finding the age at menarche to be as late as 16.5 years in 1840. They also observed a similar decline (2 months per decade) in US reports from 1877 to 1947. A more recent study assessing a cohort of 94,170 British women found a decline in age at menarche from 13.5 for girls born between 1908 and 1919 to 12.3 for girls born between 1990 and 1993 (84). This decline in age at menarche has also been observed in macaque colonies (87, 88)

indicating that rather than being an effect of evolution, the decrease is due to environmental influences. Both the earlier and more recent human studies noticed that the rate of decline in age at menarche slowed in 1940s and has now been fairly consistent (average of 12 years of age) over the past 70 years. It is believed that this plateau in menarcheal age is due to improved nutrition and life quality. This is an important finding as the breast cancer incidence has continued to increase rapidly over the past century suggesting that early age at menarche is not the major reproductive factor influencing breast cancer incidence.

Factors that have been shown to affect the age at menarche include gestational exposure to smoke (89), diet (90), psychological state (91, 92), and BMI (93–95). The effect of BMI on age at menarche has not only been supported by numerous epidemiological findings but has also been shown experimentally in rhesus monkeys (87) and confirmed by genome-wide sequencing (96, 97). In one sequencing study, 30 new loci associated with age at menarche were identified, most of which have no clear function individually, but pathway analysis classified them into two groups, lipid metabolism and gene expression/cellular growth (96). While poor dietary choices contributing to BMI is considered an environmental factor influencing age at menarche, the effect of BMI on age at menarche has also been shown to be due to heritable factors (98). Indeed, excessive maternal weight gain during gestation has been shown to lower the age at menarche in daughters (99, 100). In concert with this, small-scale studies have shown that increased gestational weight gain leads to greater chance of obesity in adolescent offspring (101, 102), which then is known to influence the age at menarche (89, 93–97). Cumulatively, these data indicate that alterations during critical developmental points can actually determine a daughter's weight which then influences her age at menarche and then in turn her breast cancer risk later in life.

It is unexpected that genome-wide sequencing studies did not find a strong association of estrogen-regulated genes and pathways with age at menarche, as was shown in the age at menopause studies (67, 96, 97). Certainly, menarche begins in response to ovarian hormones including estrogen, and higher levels of urinary estrogens have been observed in girls experiencing precocious menarche (103). This may be a key link to the influence age at menarche has on breast cancer risk, as if higher estrogen levels correlate with earlier age at menarche, it may be that the increase in breast cancer risk associated with younger ages at menarche is estrogen driven.

Despite the identification of candidate genes involved in the timing of menarche, experimental work to define the mechanism/s underlying pubertal timing has been limited. Most studies have been restricted to exploring the effects of environmental determinants such as exposure to seasonal changes (although the effect on age at menarche is thought to be a by-product of changes in growth), diet, and social status. This is due to the required use of macaques and non-human primates who also undergo a defined menarche (104). Of the 19 single nucleotide polymorphisms that have been associated with age at menarche, only two have also been associated with breast cancer risk (71), and thus a lot is still unknown about both why a women undergoes menarche when she does and why this affects her breast cancer risk.

AGE AT MENARCHE IS MORE INFLUENTIAL THAN AGE AT MENOPAUSE ON BREAST CANCER RISK

The observation that lengthening the reproductive life of a woman, either by an earlier menarche or later menopause, increases the risk of breast cancer would suggest that the overall duration of the exposure to estrogen is underlying the risk. However, a recent meta-analysis of reproductive events and breast cancer risk has found that age at menarche may be more of a deciding factor on the risk than age at menopause (10).

It is common practice for all epidemiological studies assessing reproductive factors on breast cancer risk to consider both menarche and menopause. This is due in large part to the earliest epidemiological studies identifying an association between reproductive timing and breast cancer risk (15, 55). Reanalysis of these landmark studies using modern statistics confirmed the early associations and also assessed whether age at menarche or age at menopause had a greater influence on breast cancer risk. This report showed inconsistent effects of age at menarche but very consistent effect of age at menopause where younger age at menopause reduces risk of breast cancer across two cohorts of women (27). A similar finding was also reported a decade earlier finding age at menopause a greater influence on breast cancer risk among parous women (105). However, since these reports, the Collaborative group on Hormonal Factors in Breast Cancer published a meta-analysis of 117 previously reported epidemiological studies showing that while later age at menopause does increase the risk of breast cancer, each year earlier at menarche increases breast cancer risk more than each year later at menopause (10). The meta-analysis included 425,055 women (118,964 cases versus 306,091 controls) providing it with sufficiently more power than the earlier work with a maximum of 1,000 cases and controls. The Collaborative group on Hormonal Factors in Breast Cancer also noted that there is no relationship between age and menarche and age at menopause, in that an earlier age at menarche does not influence the age at menopause and *vice versa*. This has been identified in a number of epidemiological reports with Forman and colleagues meta-analysis observing that of 36 studies investigating age at menarche and menopause, just 12 found a significant association between the 2 (60). This lack of an association has also more recently been supported by genome-wide sequencing analysis (70).

These findings contradict earlier theories that the influence of age at menarche and menopause on breast cancer risk was simply due to the duration of exposure to cycling ovarian hormones. Instead, it seems that the timing of the first exposure of the mammary gland to cyclic hormones sets up a developmental program that has consequences for breast cancer risk later in life.

ABERRANT HORMONE EXPOSURE IS MORE INFLUENTIAL IN THE YOUNG, RATHER THAN OLD MAMMARY GLAND

Aberrant hormone exposures *via* clinical administration or natural exposure are known to increase the risk of breast cancer.

Elevated endogenous hormones increase breast cancer risk; dizygotic twins can be exposed to up to two times the maternal estrogen levels that single pregnancies experience (106). In line with this studies have reported increased breast cancer risk in women later in life who belong to a dizygotic twin pair (106, 107).

Exogenous hormone exposure also modulates breast cancer risk with *in utero* exposure to synthetic estrogens, combined OCP use in young women, and HRT in postmenopausal women all increasing breast cancer risk. Maternal use of synthetic estrogen diethylstilbestrol, which was widely prescribed in 1940–1960s to prevent pregnancy complications, has been shown to significantly increase the risk of breast cancer in offspring (108, 109), but not until after the age of 40. Combined OCP use (estrogen + progestin) increases the risk of breast cancer (12, 110, 111) and like early age at menarche, the younger a woman is at the start of use, the higher her risk of breast cancer. One large meta-analysis reporting on 54 studies including 53,279 cases and 100,239 controls observed an RR of 1.6 (SD 0.142, $p = 0.0001$) for women who began OCP use before the age of 17 compared to an RR 1.2 (SD 0.047) in women commencing treatment after 22 years of age. The longer the duration of OCP also further increases the risk of breast cancer (112, 113). However, unlike age at menarche and *in utero* aberrant hormonal exposures, the increased risk of breast cancer from OCP is not lifelong and disappears between 4 and 10 years after use ceases (12, 110–113). Similarly, HRT use (estrogen and progestogens) in postmenopausal women increases the risk of breast cancer [adjusted RR = 2.0 (95% CI 1.8–2.12)] (114) and the risk increases with longer duration of use (11, 115). Like OCP use, the increased risk observed during HRT returns to baseline levels within 1–5 years (11, 114) but unlike OCP use, earlier age at first use is not associated with a higher increase in incidence.

Very few studies have been performed to experimentally explore the stimulatory effect of exogenous hormonal exposure on the normal mammary gland. In regard to HRT, as mentioned, rodent studies are complicated by the fact that rodents do not undergo natural menopause. Despite this, two studies have been performed assessing the effects of either estrogen alone (76) or estrogen in combination with progesterone (116) and both found a stimulation in proliferation in the mammary gland. However, estrogen alone does not increase the risk of breast cancer (117) and thus these findings cannot be readily applied to the epidemiological studies in women. Additionally, while estrogen combined with synthetic progestins is known to increase the risk of breast cancer, some studies have shown that estrogen with micronized progesterone does not (118, 119). Using macaques one group investigated the benefits of using micronized progesterone rather than synthetic progestins in HRT (120). They found increased lobular and ductal proliferation in those who received estrogen plus progestin compared to those receiving estrogen plus micronized progesterone. Transcriptional profiling of mammary tissue isolated from the two treatment groups showed a significant upregulation in the epidermal growth factor receptor/HER2 pathway and increased expression of proto-oncogene *c-MYC* (120). They did not see any changes in genes involved in ER signaling, which was unexpected given HRT increases the incidence of ER+ breast cancer. As these studies were performed in ovariectomized monkeys, their finding—while a significant

advancement in our understanding of how aberrant synthetic hormonal exposure increases breast cancer risk—can only be applied to epidemiological findings on HRT and breast cancer risk, not OCP. To date, there have been no experimental studies on the effects of estrogen and progesterin on normal mammary gland activity in ovary-intact mice.

Cumulatively, the epidemiological studies assessing aberrant hormonal exposures again point to the young mammary gland as being the most susceptible to hormone fluctuations and breast cancer risk modulation.

WHAT IS UNIQUE ABOUT THE YOUNG MAMMARY GLAND THAT MAKES IT SO SUSCEPTIBLE TO CANCER INDUCTION AND PROTECTION?

The fact that two crucial reproductive events, menarche and young age at parity, have the greatest effect on lifetime breast cancer risk suggests that the young mammary gland represents a crucial window in tumorigenic susceptibility. Why this is the case is less clear. Based on the epidemiological evidence for this, a few hypotheses have been generated, but again few have been tested experimentally, and this work is largely restricted to rodent models.

Mammary stem cells were originally proposed to play a role in the increased susceptibility to carcinogens in the young mammary gland. Russo and colleagues reported morphologically distinct structures within the mammary gland at different stages of transformation sensitivity, as ascertained through exposure to chemical carcinogens (48–50). Terminal end buds (TEBS) are club-like structures that facilitate the invasion of the mammary tree through the mammary fat pad in response to the onset of estrogen signaling at puberty (121). Once the buds reach the edges of the fat pad, they regress. Russo and colleagues quantitated the number and size of the TEBS and related the numbers to timeframes of susceptibility to the chemical carcinogen 7,12-dimethylbenz(*a*)anthracene (50). They showed that tumor incidence was highest when the TEBS number and density was highest (49, 122). As it had been previously suggested that TEBS house mammary stem cells (123), they concluded that the density and number meant more mammary stem cells and thus a larger pool of transformation-sensitive cells. These data are compelling, however, support for mammary stem cells being housed or even enriched in the TEBS is conflicting (124–127). Furthermore, many groups have shown that mammary stem cells are not highest in number in the young mammary gland, but rather accumulate with age (127–129).

It has to also be considered that the high proliferative index of the mammary gland at puberty puts the cells at risk of obtaining and perpetuating deleterious mutations. As mentioned above, the pubertal mammary glands of rodents have a high content of TEBS, and it has been shown by several groups that the cells within these TEBS are highly mitotic (124, 130–133). During the process of the cell cycle, many checkpoints are in place to ensure the integrity of the DNA to be replicated and divided before mitosis can be completed. Should an error in

DNA replication arise, DNA repair pathways are activated with varying levels of efficiency (134). Homologous recombination of double-stranded DNA breaks is considered the most effective repair mechanism, while non-homologous end-joining, although faster, is more prone to errors. Whether a normal cell chooses homologous recombination or non-homologous end joining to repair double-stranded DNA breaks is cell-cycle stage-specific (135–137). Homologous recombination is preferred for repair during S and G2/m phases as the machinery and DNA repair template required for homologous recombination are readily at hand. However, in the case of rapid proliferation, such as that seen at the onset of puberty in response to estrogen signaling (138), DNA replication stress occurs which may result in excessive amounts of homologous recombination. Ironically, this can lead to more mutations if misalignments (which are an infrequent but potentially detrimental consequence of homologous recombination) occur (139, 140). DNA replication stress can also lead to the selection of error-prone DNA damage repair mechanisms (141), and sometimes no repair at all. This results in potentially oncogenic mutations being passed onto daughter cells that then in turn may perpetuate the accumulation of more deleterious mutations. So, another hypothesis is that the earlier the surge of estrogen signaling at puberty, the earlier the start of rapid proliferation of mammary cells to generate the ductal tree and the more time the mammary gland has to accumulate these mutations that ultimately lead to tumor formation. Why this accumulation in mutations takes decades to reach a critical point at which they develop into tumors is unclear.

Switching cells from a proliferative program to a differentiation pathway is a common therapeutic avenue to prevent further growth of tumors (142–144). Pregnancy is the first time the mammary gland terminally differentiates. In the human this involves the conversion of immature type 1 mammary lobules (similar to the rodent TEBS) to the more differentiated type 3 lobules, while the acinar milk-producing units are considered a transient type 4 that arise briefly to facilitate lactation (145). Thus, parous mammary glands are comprised mostly of type 3 lobules, while nulliparous mammary gland are predominantly made up of type 1 lobules, although they do contain a small number of type 2 and even sometimes type 3 lobules. Compared to type 1 lobules, type 3 lobules are relatively growth quiescent and thus not contributing to potentially deleterious proliferative-induced DNA mutations. Therefore, should a woman undergo the terminal differentiation required for pregnancy at a young age, rather than remaining in a state of prolonged proliferation, her mammary tissue will be induced to become prematurely growth quiescent, and protected from oncogenic transformation.

CONCLUSION AND FUTURE PERSPECTIVES

Overall epidemiological studies have revealed that age at menarche is a stronger determinant of breast cancer risk than age at menopause. Despite this, the trend for a decline in age at menarche has not been steadily changing while breast cancer incidence has continued to rise, indicating that age at menarche

is not likely to be fueling the increase in breast cancer cases. Furthermore, one questions why if the age at menarche and age at menopause influence breast cancer risk, why are so few of the gene polymorphisms associated with these reproductive factors also related to breast cancer? Together the studies indicate that estrogen exposure is not the underlying link between menarche/menopause and breast cancer (because age at menarche has more of an influence). The work to date indicates that they influence breast cancer risk in different ways with menopause timing likely to be hormonally driven, whereas age at menarche might influence breast cancer risk indirectly through BMI. Increasing epidemiological evidence for these theories is emerging but functional studies are lacking.

By contrast, childbearing trends are mirroring breast cancer incidence. Increasing numbers of women today remain nulliparous, women are having fewer children and a quarter of new mothers are delaying the start of childbearing (2, 36, 37). These reproductive changes over the last century correlate with the increased breast cancer incidence over the same period. Functional studies have been performed using rodent models to show that the protection afforded by parity against breast cancer is hormonally driven and may involve mammary stem cells (53, 146), but are still yet to delineate the exact mechanisms of how undergoing a full-term pregnancy equips the mammary gland with protection against carcinogenesis.

The studies reviewed herein show that age at menarche and timing of pregnancy have the greatest influence on breast cancer

risk indicating that this early window of life is the most sensitive. The protection afforded by parity does not affect all women and unfortunately we have no way to identify those who are protected by childbearing. It is intriguing to postulate that the women with the potential to receive the protective effects of parity may be the same women who are most at risk of a carcinogenic insult. It may be that their mammary glands are more sensitive to developmental programming, be it protective or detrimental. The important questions remaining are can we predict who these women are, and can we use this information to develop preventive therapeutics? Through further functional validation of the genetic pathways involved in age at menarche, age at menopause and parity, the field may step a little closer to understanding these complex reproductive systems and their effects on breast cancer risk.

AUTHOR CONTRIBUTIONS

The authors conceived and assisted in the writing of this review article.

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REFERENCES

1. AIHW. *Breast Cancer in Australia: An Overview. Cancer Series No. 71. Cat. No. CAN 67*. Canberra: Australian Institute of Health and Welfare & Australasian Associated of Cancer Registries (2012).
2. Australian Institute of Health and Welfare, National Breast and Ovarian Cancer Centre. *Breast Cancer in Australia: An Overview*. 50th ed. Canberra: AIHW (2009).
3. Howlander N, Noone A, Krapcho M, Miller D, Bishop K, Altekruse S, et al. *SEER Cancer Statistics Review, 1975-2013*. Bethesda, MD: National Cancer Institute (2016).
4. Cancer Research UK. *Breast Cancer Statistics [Online]*. Cancer Research UK (2016).
5. Youlender DR, Cramb SM, Yip CH, Baade PD. Incidence and mortality of female breast cancer in the Asia-Pacific region. *Cancer Biol Med* (2014) 11:101–15. doi:10.7497/j.issn.2095-3941.2014.02.005
6. Li CI, Daling JR, Malone KE. Incidence of invasive breast cancer by hormone receptor status from 1992 to 1998. *J Clin Oncol* (2003) 21:28–34. doi:10.1200/JCO.2003.03.088
7. Rosenberg PS, Barker KA, Anderson WF. Estrogen receptor status and the future burden of invasive and in situ breast cancers in the United States. *J Natl Cancer Inst* (2015) 107:djv159. doi:10.1093/jnci/djv159
8. Bigaard J, Stahlberg C, Jensen MB, Ewertz M, Kroman N. Breast cancer incidence by estrogen receptor status in Denmark from 1996 to 2007. *Breast Cancer Res Treat* (2012) 136:559–64. doi:10.1007/s10549-012-2269-0
9. Glass AG, Hoover RN. Rising incidence of breast cancer: relationship to stage and receptor status. *J Natl Cancer Inst* (1990) 82:693–6. doi:10.1093/jnci/82.8.693
10. Collaborative Group on Hormonal Factors in Breast Cancer. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *Lancet Oncol* (2012) 13:1141–51. doi:10.1016/S1470-2045(12)70425-4
11. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer. *Lancet* (1997) 350:1047–59. doi:10.1016/S0140-6736(97)08233-0
12. Althuis MD, Brogan DR, Coates RJ, Daling JR, Gammon MD, Malone KE, et al. Hormonal content and potency of oral contraceptives and breast cancer risk among young women. *Br J Cancer* (2003) 88:50–7. doi:10.1038/sj.bjc.6600691
13. Ma H, Henderson KD, Sullivan-Halley J, Duan L, Marshall SF, Ursin G, et al. Pregnancy-related factors and the risk of breast carcinoma in situ and invasive breast cancer among postmenopausal women in the California Teachers Study cohort. *Breast Cancer Res* (2010) 12:R35. doi:10.1186/bcr2589
14. Ramazzini B. *De Morbis Artificum (Diseases of Workers)*. Chicago: University of Chicago Press (1940).
15. Lane-Clayton J. *A Further Report on Cancer of the Breast with Special Reference to its Associated Antecedent Conditions. Reports on Public Health and Medical Subjects No. 32*. London: HMSO (1926).
16. Fraumeni JF, Lloyd JW, Smith EM, Wagoner JK. Cancer mortality among nuns: role of marital status in etiology of neoplastic disease in women. *J Natl Cancer Inst* (1969) 42:455–68.
17. Righi S. Statistical facts about cancers on which Doctor Righi-Stern based his contribution to the Surgeons' Subgroup of the IV Congress of the Italian Scientists on 23 September 1842 (translation). *Stat Med* (1987) 6:881–4. doi:10.1002/sim.4780060803
18. Albrektsen G, Heuch I, Hansen S, Kvale G. Breast cancer risk by age at birth, time since birth and time intervals between births: exploring interaction effects. *Br J Cancer* (2005) 92:167–75. doi:10.1038/sj.bjc.6602302
19. MacMahon B, Cole P, Lin TM, Lowe CR, Mirra AP, Ravnihar B, et al. Age at first birth and breast cancer risk. *Bull World Health Organ* (1970) 43:209–21.
20. Ursin G, Bernstein L, Lord S, Karim R, Deapen D, Press M, et al. Reproductive factors and subtypes of breast cancer defined by hormone receptor and histology. *Br J Cancer* (2005) 93:364–71. doi:10.1038/sj.bjc.6602712
21. Ma H, Bernstein L, Pike MC, Ursin G. Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: a

- meta-analysis of epidemiological studies. *Breast Cancer Res* (2006) 8:R43. doi:10.1186/bcr1525
22. Anderson KN, Schwab RB, Martinez ME. Reproductive risk factors and breast cancer subtypes: a review of the literature. *Breast Cancer Res Treat* (2014) 144:1–10. doi:10.1007/s10549-014-2852-7
 23. Tamimi RM, Colditz GA, Hazra A, Baer HJ, Hankinson SE, Rosner B, et al. Traditional breast cancer risk factors in relation to molecular subtypes of breast cancer. *Breast Cancer Res Treat* (2012) 131:159–67. doi:10.1007/s10549-011-1702-0
 24. Gaudet MM, Press MF, Haile RW, Lynch CF, Glaser SL, Schildkraut J, et al. Risk factors by molecular subtypes of breast cancer across a population-based study of women 56 years or younger. *Breast Cancer Res Treat* (2011) 130:587–97. doi:10.1007/s10549-011-1616-x
 25. Chen L, Li CL, Tang MT, Porter P, Hill DA, Wiggins CL, et al. Reproductive factors and risk of luminal, HER2-overexpressing, and triple-negative breast cancer among multiethnic women. *Cancer Epidemiol Biomarkers Prev* (2016) 25:1297–304. doi:10.1158/1055-9965.EPI-15-1104
 26. Layde PM, Webster LA, Baughman AL, Wingo PA, Rubin GL, Ory HW, et al. The independent associations of parity, age at first full term pregnancy, and duration of breastfeeding with the risk of breast cancer. *J Clin Epidemiol* (1989) 42:963–73. doi:10.1016/0895-4356(89)90161-3
 27. Press DJ, Pharoah P. Risk factors for breast cancer: a reanalysis of two case-control studies from 1926 and 1931. *Epidemiology* (2010) 21:566–72. doi:10.1097/EDE.0b013e3181e08eb3
 28. Henderson BE, Powell D, Rosario I, Keys C, Hanisch R, Young M, et al. An epidemiologic study of breast cancer. *J Natl Cancer Inst* (1974) 53:609–14. doi:10.1093/jnci/53.3.609
 29. Suh JS, Yoo KY, Kwon OJ, Yun IJ, Han SH, Noh DY, et al. Menstrual and reproductive factors related to the risk of breast cancer in Korea. Ovarian hormone effect on breast cancer. *J Korean Med Sci* (1996) 11:501–8. doi:10.3346/jkms.1996.11.6.501
 30. Hirose K, Tajima K, Hamajima N, Inoue M, Takezaki T, Kuroishi T, et al. A large-scale, hospital-based case-control study of risk factors of breast cancer according to menopausal status. *Jpn J Cancer Res* (1995) 86:146–54. doi:10.1111/j.1349-7006.1995.tb03032.x
 31. Kato I, Miura S, Kasumi F, Iwase T, Tashiro H, Fujita Y, et al. A case-control study of breast cancer among Japanese women: with special reference to family history and reproductive and dietary factors. *Breast Cancer Res Treat* (1992) 24:51–9. doi:10.1007/BF01832358
 32. Tamakoshi K, Yatsuya H, Wakai K, Suzuki S, Nishio K, Lin Y, et al. Impact of menstrual and reproductive factors on breast cancer risk in Japan: results of the JACC study. *Cancer Sci* (2005) 96:57–62. doi:10.1111/j.1349-7006.2005.00010.x
 33. Lord SJ, Bernstein L, Johnson KA, Malone KE, McDonald JA, Marchbanks PA, et al. Breast cancer risk and hormone receptor status in older women by parity, age of first birth, and breastfeeding: a case-control study. *Cancer Epidemiol Biomarkers Prev* (2008) 17:1723–30. doi:10.1158/1055-9965.EPI-07-2824
 34. Lambe M, Hsieh CC, Chan HW, Ekbohm A, Trichopoulos D, Adami HO. Parity, age at first and last birth, and risk of breast cancer: a population-based study in Sweden. *Breast Cancer Res Treat* (1996) 38:305–11. doi:10.1007/BF01806150
 35. Kauppila A, Kyyronen P, Hinkula M, Pukkala E. Birth intervals and breast cancer risk. *Br J Cancer* (2009) 101:1213–7. doi:10.1038/sj.bjc.6605300
 36. Livingston G, Cohn D. *U.S. Birth Rate Falls to a Record Low; Decline is Greatest Among Immigrants*. Washington, DC: Pew Research Center, Social & Demographic Trends (2012).
 37. Hilder L, Zhichao Z, Parker M, Jahan S, Chambers G. *Australia's Mothers and Babies 2012*. Canberra: Perinatal Statistics (2014).
 38. Hadjisavvas A, Loizidou MA, Middleton N, Michael T, Papachristoforou R, Kakouri E, et al. An investigation of breast cancer risk factors in Cyprus: a case control study. *BMC Cancer* (2010) 10:447. doi:10.1186/1471-2407-10-447
 39. Lambertini M, Santoro L, Del Mastro L, Nguyen B, Livraghi L, Ugolini D, et al. Reproductive behaviors and risk of developing breast cancer according to tumor subtype: a systematic review and meta-analysis of epidemiological studies. *Cancer Treat Rev* (2016) 49:65–76. doi:10.1016/j.ctrv.2016.07.006
 40. Beral V; Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50,302 women with breast cancer and 96,973 women without the disease. *Lancet* (2002) 360:187–95. doi:10.1016/S0140-6736(02)09454-0
 41. Huo D, Adebamowo CA, Ogundiran TO, Akang EE, Campbell O, Adenipekun A, et al. Parity and breastfeeding are protective against breast cancer in Nigerian women. *Br J Cancer* (2008) 98:992–6. doi:10.1038/sj.bjc.6604275
 42. NHMRC. *Dietary Guidelines for Children and Adolescents in Australia*. Canberra: National Health and Medical Research Council (2003).
 43. ABS. *Australian Health Survey: Health Service Usage and Health Related Actions, 2011-12. Cat. No. 4364.0.55.002*. Canberra: Australian Bureau of Statistics (2013).
 44. Ma H, Wang Y, Sullivan-Halley J, Weiss L, Burkman RT, Simon MS, et al. Breast cancer receptor status: do results from a centralized pathology laboratory agree with SEER registry reports? *Cancer Epidemiol Biomarkers Prev* (2009) 18:2214–20. doi:10.1158/1055-9965.EPI-09-0301
 45. Elshay WM. The protective effect of longer duration of breastfeeding against pregnancy-associated triple negative breast cancer. *Oncotarget* (2016) 7:53941–50. doi:10.18632/oncotarget.9690
 46. Lim E, Wu D, Pal B, Bouras T, Asselin-Labat ML, Vaillant F, et al. Transcriptome analyses of mouse and human mammary cell subpopulations reveals multiple conserved genes and pathways. *Breast Cancer Res* (2010) 12:R21. doi:10.1186/bcr2560
 47. Cardiff RD. The biology of mammary transgenes: five rules. *J Mammary Gland Biol Neoplasia* (1996) 1:61–73. doi:10.1007/BF02096303
 48. Russo J, Tait L, Russo IH. Susceptibility of the mammary gland to carcinogenesis. III. The cell of origin of rat mammary carcinoma. *Am J Pathol* (1983) 113:50–66.
 49. Russo J, Russo IH. DNA labeling index and structure of the rat mammary gland as determinants of its susceptibility to carcinogenesis. *J Natl Cancer Inst* (1978) 61:1451–9.
 50. Russo IH, Russo J. Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenz[a]anthracene. *J Natl Cancer Inst* (1978) 61:1439–49.
 51. Guzman RC, Yang J, Rajkumar L, Thordarson G, Chen X, Nandi S. Hormonal prevention of breast cancer: mimicking the protective effect of pregnancy. *Proc Natl Acad Sci U S A* (1999) 96:2520–5. doi:10.1073/pnas.96.5.2520
 52. Rajkumar L, Guzman RC, Yang J, Thordarson G, Talamantes F, Nandi S. Short-term exposure to pregnancy levels of estrogen prevents mammary carcinogenesis. *Proc Natl Acad Sci U S A* (2001) 98:11755–9. doi:10.1073/pnas.201393798
 53. Dall G, Risbridger G, Britt K. Mammary stem cells and parity-induced breast cancer protection – new insights. *J Steroid Biochem Mol Biol* (2016) 170:54–60. doi:10.1016/j.jsbmb.2016.02.018
 54. Katz TA, Liao SG, Palmieri VJ, Dearth RK, Pathiraja TN, Huo Z, et al. Targeted DNA methylation screen in the mouse mammary genome reveals a parity-induced hypermethylation of Igf1r that persists long after parturition. *Cancer Prev Res* (2015) 8:1000–9. doi:10.1158/1940-6207.CAPR-15-0178
 55. Wainwright JM. A comparison of conditions associated with breast cancer in Great Britain and America. *Am J Cancer* (1931) 15:2610–45.
 56. Gao Y-T, Shu X-O, Dai Q, Potter JD, Brinton LA, Wen W, et al. Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai breast cancer study. *Int J Cancer* (2000) 87:295–300. doi:10.1002/1097-0215(20000715)87:2<295::AID-IJC23>3.0.CO;2-7
 57. Reeves GK, Pirie K, Green J, Bull D, Beral V; Million Women Study Collaborators. Reproductive factors and specific histological types of breast cancer: prospective study and meta-analysis. *Br J Cancer* (2009) 100:538–44. doi:10.1038/sj.bjc.6604853
 58. O'Brien KM, Sun J, Sandler DP, Deroo LA, Weinberg CR. Risk factors for young-onset invasive and in situ breast cancer. *Cancer Causes Control* (2015) 26:1771–8. doi:10.1007/s10552-015-0670-9
 59. Sisti JS, Collins LC, Beck AH, Tamimi RM, Rosner BA, Eliassen AH. Reproductive risk factors in relation to molecular subtypes of breast cancer: results from the nurses' health studies. *Int J Cancer* (2016) 138:2346–56. doi:10.1002/ijc.29968
 60. Forman MR, Mangini LD, Thelus-Jean R, Hayward MD. Life-course origins of the ages at menarche and menopause. *Adolesc Health Med Ther* (2013) 4:1–21. doi:10.2147/AHMT.S15946
 61. Van Asselt KM, Kok HS, Pearson PL, Dubas JS, Peeters PH, Te Velde ER, et al. Heritability of menopausal age in mothers and daughters. *Fertil Steril* (2004) 82:1348–51. doi:10.1016/j.fertnstert.2004.04.047

62. Murabito JM, Yang Q, Fox CS, Cupples LA. Genome-wide linkage analysis to age at natural menopause in a community-based sample: the Framingham Heart Study. *Fertil Steril* (2005) 84:1674–9. doi:10.1016/j.fertnstert.2005.05.046
63. Snieder H, Macgregor AJ, Spector TD. Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. *J Clin Endocrinol Metab* (1998) 83:1875–80. doi:10.1210/jc.83.6.1875
64. Steiner AZ, Baird DD, Kesner JS. Mother's menopausal age is associated with her daughter's early follicular phase urinary follicle-stimulating hormone level. *Menopause* (2008) 15:940–4. doi:10.1097/gme.0b013e31816429e5
65. Weel AE, Uitterlinden AG, Westendorp IC, Burger H, Schuit SC, Hofman A, et al. Estrogen receptor polymorphism predicts the onset of natural and surgical menopause. *J Clin Endocrinol Metab* (1999) 84:3146–50. doi:10.1210/jcem.84.9.5981
66. He LN, Xiong DH, Liu YJ, Zhang F, Recker RR, Deng HW. Association study of the oestrogen signalling pathway genes in relation to age at natural menopause. *J Genet* (2007) 86:269–76. doi:10.1007/s12041-007-0034-7
67. Stolk L, Perry JRB, Chasman DI, He C, Mangino M, Sulem P, et al. Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. *Nat Genet* (2012) 44:260–8. doi:10.1038/ng.1051
68. Stolk L, Zhai G, Van Meurs JB, Verbiest MM, Visser JA, Estrada K, et al. Loci at chromosomes 13, 19 and 20 influence age at natural menopause. *Nat Genet* (2009) 41:645–7. doi:10.1038/ng.387
69. He C, Kraft P, Chasman DI, Buring JE, Chen C, Hankinson SE, et al. A large-scale candidate gene association study of age at menarche and age at natural menopause. *Hum Genet* (2010) 128:515–27. doi:10.1007/s00439-010-0878-4
70. Perry JRB, Hsu Y-H, Chasman DI, Johnson AD, Elks C, Albrecht E, et al. DNA mismatch repair gene MSH6 implicated in determining age at natural menopause. *Hum Mol Genet* (2014) 23:2490–7. doi:10.1093/hmg/ddt620
71. He C, Chasman DI, Dreyfus J, Hwang SJ, Ruiter R, Sanna S, et al. Reproductive aging-associated common genetic variants and the risk of breast cancer. *Breast Cancer Res* (2012) 14:R54. doi:10.1186/bcr3155
72. Steward MM, Lee J-S, O'Donovan A, Wyatt M, Bernstein BE, Shilatifard A. Molecular regulation of H3K4 trimethylation by ASH2L, a shared subunit of MLL complexes. *Nat Struct Mol Biol* (2006) 13:852. doi:10.1038/nsmb1131
73. Won Jeong K, Chodankar R, Purcell DJ, Bittencourt D, Stallcup MR. Gene-specific patterns of coregulator requirements by estrogen receptor- α in breast cancer cells. *Mol Endocrinol* (2012) 26:955–66. doi:10.1210/me.2012-1066
74. Zou JX, Duan Z, Wang J, Sokolov A, Xu J, Chen CZ, et al. Kinesin family deregulation coordinated by bromodomain protein ANCCA and histone methyltransferase MLL for breast cancer cell growth, survival and tamoxifen resistance. *Mol Cancer Res* (2014) 12:539–49. doi:10.1158/1541-7786.MCR-13-0459
75. Den Tonkelaar I, Te Velde ER, Looman CWN. Menstrual cycle length preceding menopause in relation to age at menopause. *Maturitas* (1998) 29:115–23. doi:10.1016/S0378-5122(98)00013-9
76. Raafat AM, Hofseth LJ, Li S, Bennett JM, Haslam SZ. A mouse model to study the effects of hormone replacement therapy on normal mammary gland during menopause: enhanced proliferative response to estrogen in late postmenopausal mice. *Endocrinology* (1999) 140:2570–80. doi:10.1210/endo.140.6.6634
77. Golub MS, Collman GW, Foster PMD, Kimmel CA, Rajpert-De Meyts E, Reiter EO, et al. Public health implications of altered puberty timing. *Pediatrics* (2008) 121:S218–30. doi:10.1542/peds.2007-1813G
78. Sisti JS, Bernstein JL, Lynch CF, Reiner AS, Mellemlkjaer L, Brooks JD, et al. Reproductive factors, tumor estrogen receptor status and contralateral breast cancer risk: results from the WECARE study. *Springerplus* (2015) 4:825. doi:10.1186/s40064-015-1642-y
79. Nelson HD, Zakher B, Cantor A, Fu R, Griffin J, O'Meara ES, et al. Risk factors for breast cancer for women aged 40 to 49 years: a systematic review and meta-analysis. *Ann Intern Med* (2012) 156:635–48. doi:10.7326/0003-4819-156-9-201205010-00006
80. Ambrosone CB, Zirpoli G, Hong CC, Yao S, Troester MA, Bandera EV, et al. Important role of menarche in development of estrogen receptor-negative breast cancer in African American Women. *J Natl Cancer Inst* (2015) 107: djv172. doi:10.1093/jnci/djv172
81. Hamilton AS, Mack TM. Puberty and genetic susceptibility to breast cancer in a case-control study in twins. *N Engl J Med* (2003) 348:2313–22. doi:10.1056/NEJMoa021293
82. Braithwaite D, Moore DH, Lustig RH, Epel ES, Ong KK, Rehkopf DH, et al. Socioeconomic status in relation to early menarche among black and white girls. *Cancer Causes Control* (2009) 20:713–20. doi:10.1007/s10552-008-9284-9
83. Anderson SE, Must A. Interpreting the continued decline in the average age at menarche: results from two nationally representative surveys of U.S. girls studied 10 years apart. *J Pediatr* (2005) 147:753–60. doi:10.1016/j.jpeds.2005.07.016
84. Morris DH, Jones ME, Schoemaker MJ, Ashworth A, Swerdlow AJ. Secular trends in age at menarche in women in the UK born 1908–93: results from the Breakthrough Generations Study. *Paediatr Perinat Epidemiol* (2011) 25:394–400. doi:10.1111/j.1365-3016.2011.01202.x
85. Parent A-S, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon J-P. The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. *Endocr Rev* (2003) 24:668–93. doi:10.1210/er.2002-0019
86. Wyshak G, Frisch RE. Evidence for a secular trend in age of menarche. *N Engl J Med* (1982) 306:1033–5. doi:10.1056/NEJM198204293061707
87. Terasawa E, Kurian JR, Keen KL, Shiel NA, Colman RJ, Capuano SV. Body weight impact on puberty: effects of high-calorie diet on puberty onset in female rhesus monkeys. *Endocrinology* (2012) 153:1696–705. doi:10.1210/en.2011-1970
88. Wilen R, Naftolin F. Age, weight and weight gain in the individual pubertal female rhesus monkey (*Macaca mulatta*). *Biol Reprod* (1976) 15:356–60. doi:10.1095/biolreprod15.3.356
89. Behie AM, O'Donnell MH. Prenatal smoking and age at menarche: influence of the prenatal environment on the timing of puberty. *Hum Reprod* (2015) 30:957–62. doi:10.1093/humrep/dev033
90. Carwile JL, Willett WC, Spiegelman D, Hertzmark E, Rich-Edwards J, Frazier AL, et al. Sugar-sweetened beverage consumption and age at menarche in a prospective study of US girls. *Hum Reprod* (2015) 30:675–83. doi:10.1093/humrep/deu349
91. Chisholm JS, Quinlivan JA, Petersen RW, Coall DA. Early stress predicts age at menarche and first birth, adult attachment, and expected lifespan. *Hum Nat* (2005) 16:233–65. doi:10.1007/s12110-005-1009-0
92. Belsky J, Steinberg L, Draper P. Childhood experience, interpersonal development, and reproductive strategy: and evolutionary theory of socialization. *Child Dev* (1991) 62:647–70. doi:10.2307/1131166
93. Fernandez-Rhodes L, Demerath EW, Cousminer DL, Tao R, Dreyfus JG, Esko T, et al. Association of adiposity genetic variants with menarche timing in 92,105 women of European descent. *Am J Epidemiol* (2013) 178:451–60. doi:10.1093/aje/kws473
94. Silva IDS, De Stavola BL, Mann V, Kuh D, Hardy R, Wadsworth ME. Prenatal factors, childhood growth trajectories and age at menarche. *Int J Epidemiol* (2002) 31:405–12. doi:10.1093/intjpid/31.2.405
95. Harris MA, Prior JC, Koehoorn M. Age at menarche in the Canadian population: secular trends and relationship to adulthood BMI. *J Adolesc Health* (2008) 43:548–54. doi:10.1016/j.jadohealth.2008.07.017
96. Elks CE, Perry JR, Sulem P, Chasman DI, Franceschini N, He C, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet* (2010) 42:1077–85. doi:10.1038/ng.714
97. Perry JR, Stolk L, Franceschini N, Lunetta KL, Zhai G, McArdle PE, et al. Meta-analysis of genome-wide association data identifies two loci influencing age at menarche. *Nat Genet* (2009) 41:648–50. doi:10.1038/ng.386
98. Wang W, Zhao LJ, Liu YZ, Recker RR, Deng HW. Genetic and environmental correlations between obesity phenotypes and age at menarche. *Int J Obes (Lond)* (2006) 30:1595–600. doi:10.1038/sj.ijo.0803322
99. Deardorff J, Berry-Millett R, Rehkopf D, Luecke E, Lahiff M, Abrams B. Maternal pre-pregnancy BMI, gestational weight gain, and age at menarche in daughters. *Matern Child Health J* (2013) 17:1391–8. doi:10.1007/s10995-012-1139-z
100. Boynton-Jarrett R, Rich-Edwards J, Fredman L, Hibert EL, Michels KB, Forman MR, et al. Gestational weight gain and daughter's age at menarche. *J Womens Health (Larchmt)* (2011) 20:1193–200. doi:10.1089/jwh.2010.2517
101. Diesel JC, Bodnar LM, Day NL, Larkby CA. Childhood maltreatment and the risk of pre-pregnancy obesity and excessive gestational weight gain. *Matern Child Nutr* (2016) 12:558–68. doi:10.1111/mcn.12147
102. Rooney BL, Mathiason MA, Schauburger CW. Predictors of obesity in childhood, adolescence, and adulthood in a birth cohort. *Matern Child Health J* (2011) 15:1166–75. doi:10.1007/s10995-010-0689-1

103. Shi L, Remer T, Buyken AE, Hartmann MF, Hoffmann P, Wudy SA. Prepubertal urinary estrogen excretion and its relationship with pubertal timing. *Am J Physiol Endocrinol Metab* (2010) 299:E990–7. doi:10.1152/ajpendo.00374.2010
104. Stephens SBZ, Wallen K. Environmental and social influences on neuroendocrine puberty and behavior in macaques and other nonhuman primates. *Horm Behav* (2013) 64:226–39. doi:10.1016/j.yhbeh.2013.05.003
105. Enger SM, Ross RK, Paganini-Hill A, Bernstein L. Breastfeeding experience and breast cancer risk among postmenopausal women. *Cancer Epidemiol Biomarkers Prev* (1998) 7:365–9.
106. Braun MM, Ahlbom A, Floderus B, Brinton LA, Hoover RN. Effect of twinning on incidence of cancer of the testis, breast, and other sites (Sweden). *Cancer Causes Control* (1995) 6:519–24. doi:10.1007/BF00054160
107. Cerhan JR, Kushi LH, Olson JE, Rich SS, Zheng W, Folsom AR, et al. Twinship and risk of postmenopausal breast cancer. *J Natl Cancer Inst* (2000) 92:261–5. doi:10.1093/jnci/92.3.261
108. Palmer JR, Hatch EE, Rosenberg CL, Hartge P, Kaufman RH, Titus-Ernstoff L, et al. Risk of breast cancer in women exposed to diethylstilbestrol in utero: preliminary results (United States). *Cancer Causes Control* (2002) 13:753–8. doi:10.1023/A:1020254711222
109. Hoover RN, Hyer M, Pfeiffer RM, Adam E, Bond B, Cheville AL, et al. Adverse health outcomes in women exposed in utero to diethylstilbestrol. *N Engl J Med* (2011) 365:1304–14. doi:10.1056/NEJMoa1013961
110. Ursin G, Ross RK, Sullivan-Halley J, Hanisch R, Henderson B, Bernstein L. Use of oral contraceptives and risk of breast cancer in young women. *Breast Cancer Res Treat* (1998) 50:175–84. doi:10.1023/A:1006037823178
111. Calle EE, Heath CW Jr, Miracle-McMahill HL, Coates RJ, Liff JM, Franceschi S, et al. Breast cancer and hormonal contraceptives: further results. *Contraception* (1998) 54:1–106. doi:10.1016/S0010-7824(15)30002-0
112. Hunter DJ, Colditz GA, Hankinson SE, Malspeis S, Spiegelman D, Chen W, et al. Oral contraceptive use and breast cancer: a prospective study of young women. *Cancer Epidemiol Biomarkers Prev* (2010) 19:2496–502. doi:10.1158/1055-9965.EPI-10-0747
113. Charlton BM, Rich-Edwards JW, Colditz GA, Missmer SA, Rosner BA, Hankinson SE, et al. Oral contraceptive use and mortality after 36 years of follow-up in the Nurses' Health Study: prospective cohort study. *BMJ* (2014) 349:g6356. doi:10.1136/bmj.g6356
114. Beral V; Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* (2003) 362:419–27. doi:10.1016/S0140-6736(03)14065-2
115. Li CI, Malone KE, Porter PL, Weiss NS, Tang MT, Cushing-Haugen KL, et al. Relationship between long durations and different regimens of hormone therapy and risk of breast cancer. *JAMA* (2003) 289:3254–63. doi:10.1001/jama.289.24.3254
116. Said TK, Conneely OM, Medina D, O'Malley BW, Lydon JP. Progesterone, in addition to estrogen, induces cyclin D1 expression in the murine mammary epithelial cell, in vivo. *Endocrinology* (1997) 138:3933–9. doi:10.1210/endo.138.9.5436
117. Yang Z, Hu Y, Zhang J, Xu L, Zeng R, Kang D. Estradiol therapy and breast cancer risk in perimenopausal and postmenopausal women: a systematic review and meta-analysis. *Gynecol Endocrinol* (2017) 33:87–92. doi:10.1080/09513590.2016.1248932
118. Fournier A, Berrino F, Clavel-Chapelon F. Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study. *Breast Cancer Res Treat* (2008) 107:103–11. doi:10.1007/s10549-007-9604-x
119. Fournier A, Berrino F, Riboli E, Avenel V, Clavel-Chapelon F. Breast cancer risk in relation to different types of hormone replacement therapy in the E3N-EPIC cohort. *Int J Cancer* (2005) 114:448–54. doi:10.1002/ijc.20710
120. Wood CE, Register TC, Lees CJ, Chen H, Kimrey S, Mark Cline J. Effects of estradiol with micronized progesterone or medroxyprogesterone acetate on risk markers for breast cancer in postmenopausal monkeys. *Breast Cancer Res Treat* (2007) 101:125–34. doi:10.1007/s10549-006-9276-y
121. Smalley M, Ashworth A. Stem cells and breast cancer: a field in transit. *Nat Rev Cancer* (2003) 3:832–44. doi:10.1038/nrc1212
122. Russo J, Wilgus G, Russo IH. Susceptibility of the mammary gland to carcinogenesis: I Differentiation of the mammary gland as determinant of tumor incidence and type of lesion. *Am J Pathol* (1979) 96:721–36.
123. Williams JM, Daniel CW. Mammary ductal elongation: differentiation of myoepithelium and basal lamina during branching morphogenesis. *Dev Biol* (1983) 97:274–90. doi:10.1016/0012-1606(83)90086-6
124. Rios AC, Fu NY, Lindeman GJ, Visvader JE. In situ identification of bipotent stem cells in the mammary gland. *Nature* (2014) 506:322–7. doi:10.1038/nature12948
125. Bai L, Rohrschneider LR. s-SHIP promoter expression marks activated stem cells in developing mouse mammary tissue. *Genes Dev* (2010) 24:1882–92. doi:10.1101/gad.1932810
126. Kenney NJ, Smith GH, Lawrence E, Barrett JC, Salomon DS. Identification of stem cell units in the terminal end bud and duct of the mouse mammary gland. *J Biomed Biotechnol* (2001) 1:133–43. doi:10.1155/S1110724301000304
127. Dall GV, Vieuxseux JL, Korach KS, Arai Y, Hewitt SC, Hamilton KJ, et al. SCA-1 labels a subset of estrogen-responsive bipotential repopulating cells within the CD24⁺ CD49f^{hi} mammary stem cell-enriched compartment. *Stem Cell Reports* (2017) 8:417–31. doi:10.1016/j.stemcr.2016.12.022
128. Plaks V, Brenot A, Lawson DA, Linnemann JR, Van Kappel EC, Wong KC, et al. Lgr5-expressing cells are sufficient and necessary for postnatal mammary gland organogenesis. *Cell Rep* (2013) 3:70–8. doi:10.1016/j.celrep.2012.12.017
129. Huh SJ, Clement K, Jee D, Merlini A, Choudhury S, Maruyama R, et al. Age- and pregnancy-associated DNA methylation changes in mammary epithelial cells. *Stem Cell Reports* (2015) 4:297–311. doi:10.1016/j.stemcr.2014.12.009
130. Xiong G, Xu R. ROR α binds to E2F1 to inhibit cell proliferation and regulate mammary gland branching morphogenesis. *Mol Cell Biol* (2014) 34:3066–75. doi:10.1128/MCB.00279-14
131. Russo J, Russo IH. Influence of differentiation and cell kinetics on the susceptibility of the rat mammary gland to carcinogenesis. *Cancer Res* (1980) 40:2677–87.
132. Dulbecco R, Henahan M, Armstrong B. Cell types and morphogenesis in the mammary gland. *Proc Natl Acad Sci U S A* (1982) 79:7346–50. doi:10.1073/pnas.79.23.7346
133. Bonnette SG, Hadsell DL. Targeted disruption of the IGF-I receptor gene decreases cellular proliferation in mammary terminal end buds. *Endocrinology* (2001) 142:4937–45. doi:10.1210/endo.142.11.8500
134. Lord CJ, Ashworth A. The DNA damage response and cancer therapy. *Nature* (2012) 481:287–94. doi:10.1038/nature10760
135. Rousseau L, Etienne O, Roque T, Desmaze C, Haton C, Mouthon M-A, et al. In vivo importance of homologous recombination DNA repair for mouse neural stem and progenitor cells. *PLoS One* (2012) 7:e37194. doi:10.1371/journal.pone.0037194
136. Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. *Nat Rev Mol Cell Biol* (2008) 9:297–308. doi:10.1038/nrm2351
137. Rothkamm K, Krüger I, Thompson LH, Löbrich M. Pathways of DNA double-strand break repair during the mammalian cell cycle. *Mol Cell Biol* (2003) 23:5706–15. doi:10.1128/MCB.23.16.5706-5715.2003
138. Macias H, Hinck L. Mammary gland development. *Wiley Interdiscip Rev Dev Biol* (2012) 1:533–57. doi:10.1002/wdev.35
139. Reliene R, Bishop AJR, Schiestl RH. Involvement of homologous recombination in carcinogenesis. *Advances in Genetics* (2007) 58:67–87. doi:10.1016/S0065-2660(06)58003-4
140. Bishop AJR, Schiestl RH. Homologous recombination and its role in carcinogenesis. *J Biomed Biotechnol* (2002) 2:75–85. doi:10.1155/S1110724302204052
141. Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. *Science* (2008) 319:1352–5. doi:10.1126/science.1140735
142. Felsher DW, Bishop JM. Reversible tumorigenesis by MYC in hematopoietic lineages. *Mol Cell* (1999) 4:199–207. doi:10.1016/S1097-2765(00)80367-6
143. Jain M, Arvanitis C, Chu K, Dewey W, Leonhardt E, Trinh M, et al. Sustained loss of a neoplastic phenotype by brief inactivation of MYC. *Science* (2002) 297:102–4. doi:10.1126/science.1071489
144. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* (1996) 2:561–6. doi:10.1038/nm0596-561
145. Russo J, Mailo D, Hu YF, Balogh G, Sheriff F, Russo IH. Breast differentiation and its implication in cancer prevention. *Clin Cancer Res* (2005) 11(2 Pt 2):931s–6s.
146. Meier-Abt F, Milani E, Roloff T, Brinkhaus H, Duss S, Meyer DS, et al. Parity induces differentiation and reduces Wnt/Notch signaling ratio and

proliferation potential of basal stem/progenitor cells isolated from mouse mammary epithelium. *Breast Cancer Res* (2013) 15:R36. doi:10.1186/bcr3419

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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