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Reproductive factors, menopausal hormone therapies and primary liver cancer risk: a systematic review and dose–response meta-analysis of observational studies

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BACKGROUND: A striking gender disparity in the incidence and outcome of primary liver cancer (PLC) has been well recognized. Mounting evidence from basic research suggests that hormonal factors may be involved in the gender disparity of PLC. Whether hormonal exposures in human subjects are associated with PLC risk is largely unknown.

OBJECTIVE AND RATIONALE: Whether reproductive factors and use of menopausal hormone therapies (MHTs) in women are associated with PLC risk remains controversial. We conducted this study to clarify this issue.

SEARCH METHODS: PubMed and EMBASE were searched to July, 2016 for studies published in English or Chinese. Observational studies (cohort, nested case-control and case-control) that provided risk estimates of reproductive factors, MHTs and PLC risk were eligible. The quality of included studies was determined based on the Newcastle-Ottawa quality assessment scale. Summary risk ratios (RRs) were calculated using a random-effects model. Dose–response analysis was conducted where possible.

OUTCOMES: Fifteen peer-reviewed studies, involving 1795 PLC cases and 2 256 686 women, were included. Overall meta-analyses on parity and PLC risk did not find any significant associations; however, when restricting to studies with PLC cases ≥100, increasing parity was found to be significantly associated with a decreased risk of PLC [RR for the highest versus lowest parity 0.67, 95% CI 0.52, 0.88; RR for parous versus nulliparous 0.71, 95% CI 0.53, 0.94; RR per one live birth increase 0.93, 95% CI 0.88, 0.99]. A J-shaped relationship between parity and PLC risk was identified ($P_{\text{non-linearity}} < 0.01$). Compared with never users, the pooled RRs of PLC were 0.60 (95% CI 0.37, 0.96) for ever users of MHT, 0.73 (95% CI 0.46, 1.17) for ever users of estrogen-only therapy (ET) and 0.67 (95% CI 0.45, 1.02) for ever users of estrogen–progestin therapy (EPT). The pooled RR of PLC for the oldest versus youngest category of menarcheal age was 0.50 (95% CI 0.32, 0.79). Oophorectomy was significantly associated with an increased risk of PLC (RR 2.23, 95% CI 1.46, 3.41). No significant association of age at first birth, and spontaneous or induced abortion with PLC risk was found. No meta-analysis was performed for the association of age at menopause, breastfeeding, hysterectomy, menopausal status and stillbirth with PLC risk owing to huge methodological heterogeneity and/or very limited studies.

WIDER IMPLICATIONS: Parity is associated with PLC risk in a J-shaped dose–response pattern. Late age at menarche and ever use of MHT are associated with a reduced risk of PLC, whereas there is no association of ever use of ET and EPT, age at first birth, or spontaneous and induced abortion with PLC risk. Compared to women with no history of oophorectomy, those with a history of oophorectomy are at an increased risk of PLC. Our findings provide some epidemiological support for a role of hormonal exposures in the development of PLC in women. However, these findings should be interpreted with much caution because of the limited number of studies and potential biases, and need to be validated by studies with good design and large sample size.

Key words: parity / menopausal hormone therapy / menarche / oophorectomy / abortion / meta-analysis / primary liver cancer

Introduction

Primary liver cancer (PLC) is the sixth most common cancer globally, accounting for approximately 6% of all new cancer cases (Zimmermann *et al.*, 2016). The prognosis for PLC is poor, with 5-year relative survival of about 18% in the USA (Siegel *et al.*, 2016) and age standardized 5-year relative survival of about 10% in China (Zeng *et al.*, 2015). Currently, PLC is the second leading cause of cancer-related death worldwide because of its high incidence and poor prognosis. A striking gender disparity in the incidence of PLC has been well recognized (Seton-Rogers, 2014), with males being 2–3 times more likely to develop the disease than females (McGlynn and London, 2011). Moreover, compared with female patients with PLC, male patients have shorter overall survival time (Yang *et al.*, 2014) and a higher recurrence rate (Ikeda *et al.*, 2003). Some argue that these gender differences in the incidence and outcome of PLC can be attributable to a higher prevalence of cigarette smoking, alcohol abuse, and hepatitis B virus (HBV) or hepatitis C virus (HCV) infection in males than in females (Wands, 2007). However, similar gender disparity has also been observed in mouse models of PLC induced by chemical carcinogens (Ghebranious and Sell, 1998; Maeda *et al.*, 2005; Wands, 2007), indicating that the gender disparity in PLC cannot be completely explained by the greater prevalence of common PLC risk factors in males compared with females (McGlynn and London, 2011).

Interestingly, animal studies found that ovariectomy increased the incidence of liver tumor in diethylnitrosamine-treated female mice and administration of estrogen to diethylnitrosamine-treated male mice decreased the incidence of liver tumor (Nakatani *et al.*, 2001; Li *et al.*, 2015), indicating a protective effect of estrogen against PLC. The underlying molecular mechanisms behind this protective effect have been proposed (Liu and Liu 2014; Shi *et al.*, 2014; Montella *et al.* 2015), mainly involving the anti-inflammatory effect of estrogen (Fig. 1). Specifically, interleukin (IL)-1α released by diethylnitrosamine-induced necrotic hepatocytes can target and activate several signaling pathways in Kupffer cells (Sakurai *et al.*, 2008), including the MyD88-dependent nuclear factor −κB signaling pathway (Naugler *et al.*, 2007), signal transducer and activator of transcription 3 signaling pathway (Fan *et al.*, 2013) and CCAAT/enhancer-binding protein β signaling pathway (Liu and Liu 2014). The activation of these signaling pathways then results in the production of IL-6, which in turn promotes cancer-related inflammation, hepatic injury and compensatory proliferation of hepatocytes, finally leading to the occurrence of PLC (Naugler *et al.*, 2007; Mantovani *et al.*, 2008). Estrogen can bind to estrogen receptor α located in the nucleus of Kupffer cells, and then inhibits the production of IL-6 to exert its protective effect through interfering with the activity of the aforementioned signaling pathways (Naugler *et al.*, 2007). Estrogen can also inhibit the transcription of HBV genes (Wang *et al.*, 2012) and the production of HCV infectious particles (Hayashida *et al*, 2010), which

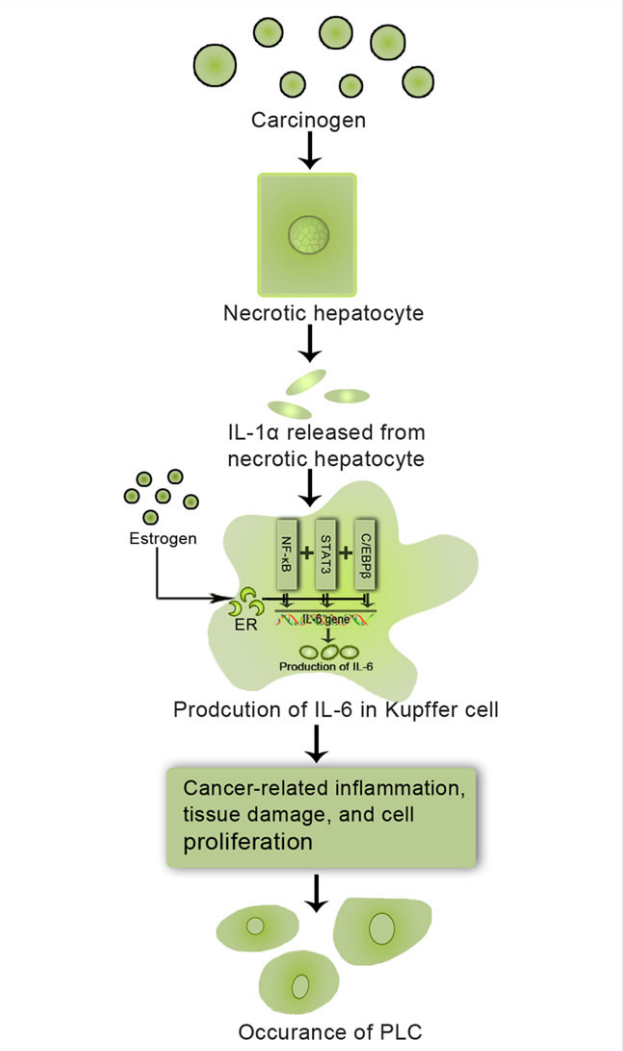

Figure 1 The molecular mechanism for the protective effect of estrogen against primary liver cancer (PLC) risk: the anti-inflammatory effect of estrogen. Estrogen can bind to estrogen receptor α, and then inhibits the production of IL-6 to exert its protective effect by decreasing the activity of transcription factors, including nuclear factor κB (NF-κB), signal transducer and activator of transcription 3 (STAT3), and CCAAT/enhancer-binding protein β (C/EBPβ).

possibly explains, at least in part, its protective role in PLC. It is noteworthy that the protective effect of estrogen can be mediated by the expression level of microRNA-18a (Liu *et al.*, 2009), and is dependent on Foxa1/2 genes (Li *et al.*, 2012). The aforementioned molecular mechanisms on the protective effect of estrogen against PLC provide an explanation for the observed gender disparity of PLC in humans. Furthermore, these results from basic research suggest that hormonal exposures in humans may be associated with PLC risk.

However, the results from observational studies on hormonal exposures and PLC risk remain controversial. For example, several studies found no association between parity and PLC risk (Hsing *et al.*, 1992; Lambe *et al.*, 1993; McGlynn *et al.*, 2015), whereas others found that parity was inversely associated with PLC risk (Yu *et al.*, 2003; Fwu *et al.*, 2009). Similarly, menopausal hormone therapy (MHT) use was found to be related to a decreased risk of PLC in several studies (Fernandez *et al.*, 2003; Yu *et al.*, 2003; McGlynn *et al.*, 2016), while no association was found in others (Persson *et al.*, 1996; McGlynn *et al.*, 2015).

To the best of our knowledge, a systematic review and meta-analysis on hormonal exposures and PLC risk is not available currently. Therefore, we conducted this study to determine whether reproductive factors and use of MHTs in women were associated with PLC risk. Moreover, we investigated the potential dose–response relationship between these exposures and PLC risk where possible.

Methods

Literature search

To identify relevant studies, a comprehensive electronic search of PubMed and EMBASE databases was performed from their inception to July 2016. Supplementary Table S1 online shows our detailed search strategy. The bibliographies of all included studies and pertinent reviews were checked carefully for identifying additional studies. We did not contact the corresponding authors to obtain extra data.

Study selection

All observational studies (cohort, nested case-control or case-control studies) published in English or Chinese were eligible for inclusion if they provided risk estimates and corresponding CIs on the association of reproductive factors (parity, age at first birth, age at menarche, age at menopause, oophorectomy, spontaneous and induced abortion, breast-feeding, hysterectomy, menopausal status and stillbirth) and MHTs [estrogen-only therapy (ET), estrogen–progestin therapy (EPT) or MHT] with PLC risk. Here, MHT refers to any hormone therapy, often of unknown or unspecified formulations. Conference abstracts were not considered in this study, considering that their results can change between meeting presentation and peer-reviewed publication. On the basis of prespecified inclusion criteria, two reviewers (J.H.C. and K.W.) first scrutinized titles and abstracts to exclude apparently ineligible studies, and then read the full text carefully to further exclude ineligible studies. Any discrepancies were resolved by discussion.

Data extraction

One reviewer (J.H.C.) extracted data through a standardized data collection form, and then another reviewer (K.W.) checked the data for accuracy. Any inconsistent results were handled by discussion. The following information was extracted: first author's family name, publication date, study location, study period, study design, study source, exposure ascertainment method, mean age, the number of PLC cases, sample size, exposure variables and their categories, the most fully adjusted risk estimates with corresponding 95% CIs and adjustment factors.

Quality assessment

Two reviewers (J.H.C. and K.W.) conducted quality assessment of included studies independently through applying the Newcastle-Ottawa quality assessment scale. This scale comprises eight items, which fall into

three domains, namely selection, comparability and outcome. After evaluating these three domains of each individual study, it could be scored a maximum of nine stars. A study earning seven or more stars was considered to be of high quality. Any disagreements on the results of quality assessment were resolved by discussion.

Statistical analysis

We pooled the risk estimate from each study using a random-effects model. Risk ratio (RR) was employed to assess the association of reproductive factors and MHTs with PLC risk. Hazard ratio (Fwu *et al.*, 2009; McGlynn *et al.*, 2015), odds ratio (Hsing *et al.*, 1992; Lambe *et al.*, 1993; Mucci *et al.*, 2001; Fernandez *et al.*, 2003; Yu *et al.*, 2003; Kanazir *et al.*, 2010; Amr *et al.*, 2014; McGlynn *et al.*, 2016) and standardized incidence ratio (Persson *et al.*,1996) were directly treated as equivalent to RR. For two studies (Stanford and Thomas, 1992; Lambe *et al.*,1993) whose authors provided risk estimates stratified by the number of live births, we pooled these stratum data through a random-effects model to approximate risk estimates for ever-parous women. Similarly, we pooled data stratified by the number of induced abortions to calculate risk estimates for women who ever had induced abortion (Stanford and Thomas, 1992). We regarded the number of full-term pregnancies as the number of live births in two studies (Yu *et al.*, 2003; Amr *et al.*, 2014). The Q statistic (a $P < 0.10$ indicating statistically significance) and the I^2 statistic (Higgins *et al.*, 2003) (an I^2 of >75.0%, 50.0–75.0% and <50% indicating substantial, moderate and low heterogeneity, respectively) were used to qualitatively and quantitatively evaluate statistical heterogeneity, respectively.

We used a random-effects dose–response meta-regression model proposed by Orsini *et al.* (2006) to calculate RRs and 95% CIs for 1-unit increment in exposure level of variables of interest. This model is based on specific exposure level, distribution of cases and person-years or controls, and adjusted RRs with 95% CIs for at least three quantitative categories. When studies reported exposure level as an interval, the midpoint of lower and upper limits was designated as the assigned dose. If the highest category was open-ended, it was assumed to share the same width as the preceding interval. If the lowest category was open-ended, the assigned dose was calculated by subtracting half of the width of the adjacent interval from the specified lowest value (Hartemink *et al.*, 2006). For one study (McGlynn *et al.*,2015), whose authors did not provide person-years by exposure level, these data were approximately estimated by multiplying the number of participants in each exposure level with the mean follow-up duration of this study. The method described by Hamling *et al.* (2008) was used to finish data conversion for two studies (Stanford and Thomas, 1992; Yu *et al.*, 2003) where the reference category was not the lowest category. A potential non-linear dose–response association of reproductive factors and MHTs with PLC risk was examined through restricted cubic spline models with three knots at the 10th, 50th and 90th percentiles (Desquilbet and Mariotti, 2010; Orsini *et al.*, 2012). A $P_{\text{non-linearity}}$ was yielded by testing the null hypothesis that the regression coefficient of the second spline equals zero (Desquilbet and Mariotti, 2010).

Three methods of sensitivity analysis were used to determine the stability of summary risk estimates, namely repeating meta-analysis with a fixed-effects model, applying diverse eligibility criteria and ignoring one study in turn. Prespecified subgroup analyses were performed to determine whether the observed associations were modified by study design, the number of PLC cases, study location, data source, and adjustment for HBV or HCV infection. We calculated a $P_{\text{interaction}}$ for the difference between subgroups through meta-regression. Note that sensitivity and subgroup analyses were only performed for parity owing to the limited studies available for the remaining exposure variables of interest, and were based on RRs for the highest versus lowest categories of parity.

We did not test publication bias with the formal statistical tests, because they have limited power when there are <10 studies. We conducted all data analyses through STATA software (version12.0, StataCorp LP, College Station, Texas, USA). Statistical significance level was set at $P < 0.05$ under a two-sided test unless otherwise specified.

Results

Literature search

The initial literature retrieval identified 4943 and 6256 citations from PubMed and EMBASE databases, respectively. A total of 7714 citations remained after removing duplicated reports. Of these remained citations, 7687 citations were excluded after scrutinizing titles and abstracts. Thirteen citations were also excluded after carefully reading the full text (Supplementary Table S2 summarizes excluded studies and corresponding reasons for exclusion). One study (Yu *et al.*, 1991) was found to be eligible for inclusion in the process of reviewing reference lists. Finally, a total of 15 studies were included in this study (Fig. 2).

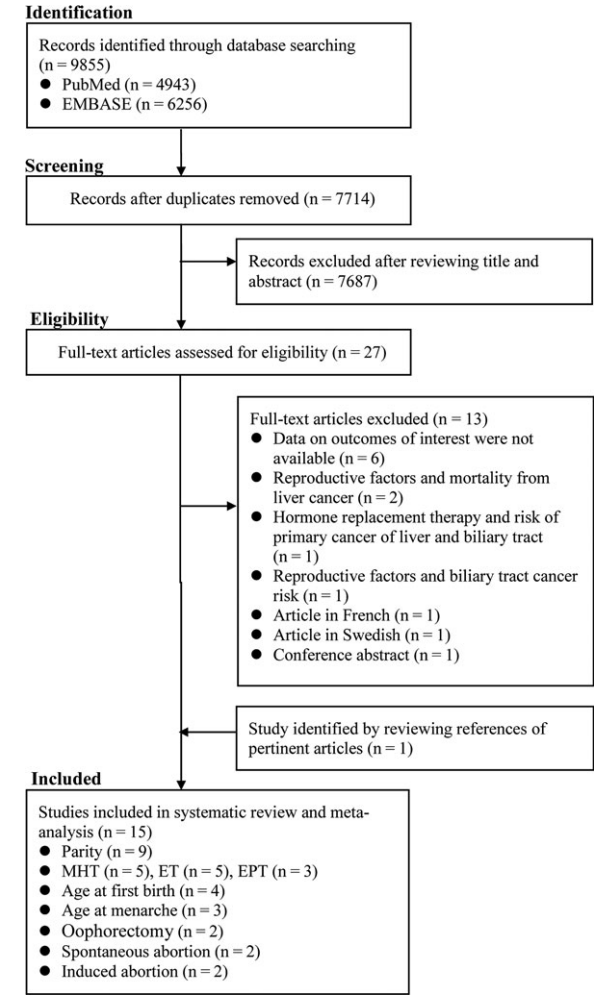

Figure 2 The flowchart of identifying relevant studies. EPT, estrogen–progestin therapy; ET, estrogen-only therapy; MHT, menopausal hormone therapy.

Study characteristics and quality assessment

The characteristics of included studies are summarized in Table I. These studies were published between 1991 (Yu et al., 1991) and 2016 (McGlynn et al., 2016). Eight studies (La Vecchia et al., 1992; Stanford and Thomas, 1992; Tavani et al., 1993; Mucci et al., 2001; Fernandez et al., 2003; Yu et al., 2003; Kanazir et al., 2010; Amr et al., 2014) recruited their participants from the hospital, whereas the remaining seven studies (Yu et al., 1991; Hsing et al., 1992; Lambe et al., 1993; Persson et al., 1996; Fwu et al., 2009; McGlynn et al., 2015, 2016) recruited participants from the general population. The methods of exposure ascertainment were somewhat varied across studies, including interviewing participants through a questionnaire (Yu et al., 1991, 2003; La Vecchia et al., 1992; Stanford and Thomas, 1992; Tavani et al., 1993; Fernandez et al., 2003; Kanazir et al., 2010; Amr et al., 2014), mailing a questionnaire to participants (Hsing et al., 1992; McGlynn et al., 2015) and using records from the national registry (Lambe et al., 1993; Persson et al., 1996; Fwu et al., 2009). The number of PLC cases ranged from 13 (Kanazir et al., 2010) to 339 (McGlynn et al., 2016), resulting in a total of 1795 PLC cases; the sample size varied from 39 (Kanazir et al., 2010) to 1 420 784 (Fwu et al., 2009), yielding a total of 2 256 686 subjects. Most included studies provided adjusted risk estimates, and most adjusted for age. As for quality assessment, nine studies were awarded ≥7 stars, and the remaining six studies were awarded 6 stars, indicating that the quality of included studies was generally good (Supplementary Tables S3 and S4).

Parity and PLC risk

Nine studies (Hsing et al., 1992; La Vecchia et al., 1992; Stanford and Thomas, 1992; Lambe et al.,1993; Yu et al., 2003; Fwu et al., 2009; Kanazir et al., 2010; Amr et al., 2014; McGlynn et al., 2015) were eligible for the association between parity and PLC risk, involving a total of 1180 PLC cases and 2 224 642 females. Overall, the summary RR of PLC for the highest versus lowest categories of parity was 1.30 (95% CI 0.74, 2.27), with substantial heterogeneity (I^2 = 74.0%, $P_{heterogeneity}$ < 0.01, 9 studies) (Fig. 3). Likewise, the summary RR of PLC for ever parity versus nulliparous was 1.42 (95% CI 0.76, 2.66), with substantial heterogeneity (I^2 = 86.4%, $P_{heterogeneity}$ < 0.01, 5 studies) (Supplementary Fig. S1). The random-effects dose–response meta-regression model showed that the RR per one live birth increase in parity was 1.00 (95% CI 0.95, 1.05, goodness-of-fit χ^2_{27} = 46.5, $P_{goodness\text{-}of\text{-}fit}$ = 0.01, 7 studies). Using restricted cubic spline model, we found a J-shaped curve for the association of parity with PLC risk ($P_{non\text{-}linearity}$ < 0.01) (Fig. 4). Compared with nulliparous women, the summary RRs were 0.87 (95% CI 0.79, 0.96) for women with one live birth, 0.80 (95% CI 0.67, 0.94) for those with two live births, 0.81 (95% CI 0.67, 0.98) for those with three live births, 0.90 (95% CI 0.73, 1.10) for those with four live births, 1.04 (95% CI 0.81, 1.33) for those with five live births and 1.23 (95% CI 0.89, 1.69) for those with six live births.

In subgroup analyses on parity and PLC risk, no evidence of modification effect by study design, study location, data source, and adjustment for HBV or HCV infection was observed (all $P_{interaction}$ > 0.05, Supplementary Table S5). However, we found strong evidence of modification effect by the number of PLC cases ($P_{interaction}$ < 0.01), with an inverse association among studies with cases ≥100 but a positive association among studies with cases <100. When restricting meta-analysis for ever parity versus nulliparous and random-effects dose–response meta-regression analysis to studies with cases ≥100 and to those with cases <100, we found similar results (Supplementary Table S6). In addition, we observed that the dose–response curve for the association of parity with PLC risk was non-linear in studies with cases ≥100 ($P_{non\text{-}linearity}$ < 0.01) but was linear in those with cases <100 ($P_{non\text{-}linearity}$ = 0.27) (Supplementary Fig. S2).

Omitting a single study in turn did not significantly alter the initial association between parity and PLC risk (Supplementary Fig. S3), with the pooled RRs ranging from 1.08 (95% CI 0.64, 1.81) (Stanford and Thomas, 1992) to 1.56 (95% CI 0.85, 2.88) (Yu et al., 2003). Repeating the analysis with a fixed-effects model and changing eligibility criteria did not significantly alter the initial association of parity with PLC risk, either (Supplementary Table S7).

MHTs and PLC risk

Seven studies (Yu et al., 1991, 2003; Tavani et al., 1993; Persson et al., 1996; Fernandez et al., 2003; McGlynn et al., 2015, 2016) were included for the association between MHT use and PLC risk, involving a total of 1031 PLC cases and 832 379 participants. Compared with never users, the combined RRs of developing PLC were 0.60 (95% CI 0.37, 0.96, I^2 = 75.5%, $P_{heterogeneity}$ < 0.01, 5 studies) for ever users of MHT, 0.73 (95% CI 0.46, 1.17, I^2 = 35.6%, $P_{heterogeneity}$ = 0.18, 5 studies) for ever users of ET and 0.67 (95% CI 0.45, 1.02, I^2 = 0.0 %, $P_{heterogeneity}$ = 0.89, 3 studies) for ever users of EPT (Fig. 5).

Age at first birth and PLC risk

Only four studies (La Vecchia et al., 1992; Stanford and Thomas, 1992; Lambe et al.,1993; McGlynn et al.,2015) were eligible for the relationship of age at first birth to PLC risk, involving a total of 543 PLC cases and 801 400 participants. On the basis of limited studies, the present meta-analysis failed to find a significant association (RR 0.71, 95% CI 0.42, 1.19, I^2 = 0.0%, $P_{heterogeneity}$ = 0.96, 4 studies) (Supplementary Fig. S4) when comparing women in the oldest age at first birth category with those in the youngest age at first birth category. There was also no significant dose–response relationship between age at first birth and PLC risk (RR for every 1 year delay 0.99, 95% CI 0.98, 1.00, goodness-of-fit χ^2_{12} = 5.82, $P_{goodness\text{-}of\text{-}fit}$ = 0.92, 4 studies). Evidence of a linear dose–response relationship of age at first birth to PLC risk was found ($P_{non\text{-}linearity}$ = 0.35, 4 studies) (Supplementary Fig. S5).

Age at menarche and PLC risk

Only three studies (Yu et al., 2003; Kanazir et al., 2010; McGlynn et al., 2015) met inclusion criteria for the relationship between age at menarche and PLC risk, involving 479 PLC cases and 800 486 women in total. The summarized RR of PLC for the oldest age group compared with the youngest age group was 0.50 (95% CI 0.32, 0.79, I^2 = 16.2%, $P_{heterogeneity}$ = 0.30, 3 studies) (Fig. 6).

Table I Characteristics of included studies on reproductive factors and MHTs and PLC risk.

Study; location	Study period	Study design	Study source	Exposure ascertainment	Mean age (y)	Cases/SS	Exposure variables	Adjustment factors
McGlynn *et al.* (2016); UK	1988–2011	Nested case-control	Population-based	Medical records	67.9	339/1657	ET, EPT, MHT	Alcohol-related disorders, aspirin, bilateral oophorectomy, BMI, diabetes, diabetes medications, HBV, HCV, hysterectomy, metabolic disorders, paracetamol use, smoking, statins
McGlynn *et al.* (2015); USA	NA	Cohort	Population-based	Questionnaire mailed to participants	NA	248/799 500	ET, EPT, MHT, age at menarche, age at menopause, age at first birth, parity, oophorectomy, hysterectomy	Age, alcohol, BMI, diabetes, education race, smoking, parent cohort study
Amr *et al.* (2014); Egypt	1999–2009	Case-control	Hospital-based	Face-to-face interview through questionnaire	46.5	132/801	Parity	Age, birthplace, education, HBV
Kanazir *et al.* (2010); Serbia	2004–2007	Case-control	Hospital-based	Face-to-face interview through questionnaire	(>60)	13/39	Age at menarche, age at menopause, parity, breastfeeding	None
Fwu *et al.* (2009); Taiwan	1983–2003	Cohort	Population-based	Records from national registry	27.5	202/1 420 784	Parity	Age at the last test, HBsAg status
Yu *et al.* (2003); Taiwan	1998–2001	Case-control	Hospital-based	Face-to-face interview through questionnaire	52.1	218/947	MHT, age at menarche, age at menopause, parity, oophorectomy, menopausal status	Age at recruitment, diabetes, hysterectomy, status, type and age of menopause
Fernandez *et al.* (2003); Italy	1983–1999	Case-control	Hospital-based	Face-to-face interview through questionnaire	(45–79)	105/7081	HT	Age, age and type of menopause, alcohol, BMI, education, smoking, study center, year of interview
Mucci *et al.* (2001); Greece	1995–1998	Case-control	Hospital-based	Face-to-face interview	NA	50/112	Age at menopause	Age, alcohol consumption, smoking,years of schooling
Persson *et al.* (1996); Sweden	1977–1991	Cohort	Population-based	Records from national registry	54.5	14/22 579	ET, EPT, MHT	None
Tavani *et al.* (1993); Italy	1984–1992	Case-control	Hospital-based	Face-to-face interview through questionnaire	59[a]	82/532	ET	Age
Lambe *et al.* (1993); Sweden	1958–1984	Nested case-control	Population-based	Records from national registry	(24–59)	133/798	Age at first birth, parity	Age
Stanford and Thomas (1992);	1979–1986	Case-control	Hospital-based	Face-to-face interview	39.8	83/679	Age at first birth, parity, spontaneous abortion, induced	Age, center, year of interview

Continued

Table I *Continued*

Study; location	Study period	Study design	Study source	Exposure ascertainment	Mean age (y)	Cases/SS	Exposure variables	Adjustment factors
China, Kenya, Philippines, Thailand				through questionnaire			abortion, breastfeeding, number of stillbirths	
La Vecchia *et al.* (1992); Italy	1984–1991	Case-control	Hospital-based	Face-to-face interview through questionnaire	NA[b]	79/423	Age at first birth, age at menopause, parity, spontaneous abortion, induced abortion	Age, alcohol consumption, education, history of hepatitis, oral contraceptive use
Hsing *et al.* (1992); USA	1985–1986	Case-control	Population-based	Questionnaire mailed to participants	NA	72/671	Parity	Age at death, duration of oral contraceptive use, race
Yu *et al.* (1991); USA	1984–1990	Case-control	Population-based	Interview through questionnaire	59.1	25/83	ET	Duration of oral contraceptive use

EPT, estrogen–progestin therapy; ET, estrogen therapy; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; MHTs, menopausal hormone therapies; NA, not available; PLC, primary liver cancer; SS, sample size; y, year.
[a]Median value.
[b]The median age for cases and controls was 57 and 58 years, respectively.

Age at menopause and PLC risk

Five studies (La Vecchia *et al.*, 1992; Mucci *et al.*, 2001; Yu *et al.*, 2003; Kanazir *et al.*, 2010; McGlynn *et al.*, 2015) were eligible for the association between age at menopause and PLC risk, involving a sum of 608 PLC cases and 801 021 subjects. Considering the huge methodological heterogeneity across studies, we did not perform a meta-analysis for this association. Supplementary Table S8 summarizes included studies on age at menopause and PLC risk. Overall, the results derived from these 5 studies are inconclusive. A large cohort study (McGlynn *et al.*, 2015) revealed no association between age at menopause and the risk of hepatocellular carcinoma (HCC), which is in agreement with the findings from a case-control study (La Vecchia *et al.*, 1992). Two case-control studies (Yu *et al.*, 2003; Kanazir *et al.*, 2010) showed that early age at menopause increased the risk of HCC, whereas another case-control study (Mucci *et al.*, 2001) showed that late age at menopause increased the risk of HCC.

Other hormonal exposures and PLC risk

In addition to the aforementioned hormonal exposures, we also investigated the association between other hormonal exposures and PLC risk, including oophorectomy (unilateral or bilateral), spontaneous and induced abortion, breastfeeding, hysterectomy, menopausal status and stillbirth (Supplementary Table S9 and Supplementary Fig. S6). Overall, there were very limited studies evaluating PLC risk in relation to these hormonal exposures. On the basis of these studies, oophorectomy was found to be significantly associated with an increased risk of PLC (RR 2.23, 95% CI 1.46, 3.41, I^2 = 0.0%, $P_{heterogeneity}$ = 0.50, 2 studies), whereas no significant association of spontaneous or induced abortion with PLC risk was found (RR for spontaneous abortion 1.10, 95% CI 0.71, 1.70, I^2 = 0.0%, $P_{heterogeneity}$ = 0.41, 2 studies; RR for induced abortion 1.27, 95% CI 0.76, 2.14, I^2 = 0.0%, $P_{heterogeneity}$ = 0.48, 2 studies) (Supplementary Fig. S6).

Discussion

Findings from the highest versus lowest meta-analysis, ever versus never meta-analysis and the dose–response meta-regression analysis consistently revealed no association between parity and PLC risk. However, when restricting these analyses to studies with PLC cases ≥100, increasing parity was consistently found to be associated with a decreased risk of PLC. Based on all included studies or those with PLC cases ≥100, a non-linear dose–response analysis identified a J-shaped curve for the association of parity with PLC risk. Ever use of MHT was related to a reduced risk of PLC, whereas ever use of ET and EPT was not related to PLC risk. On the basis of limited studies, females in the oldest category of menarcheal age were found to be at lower risk of PLC than those in the youngest category of menarcheal age; however, as for age at first birth and PLC risk, there was no significant risk difference between females in the oldest age category and those in the youngest age category.

In the present study, we observed substantial heterogeneity across studies for the association between parity and PLC risk. As indicated by our subgroup analyses, the number of PLC cases of included

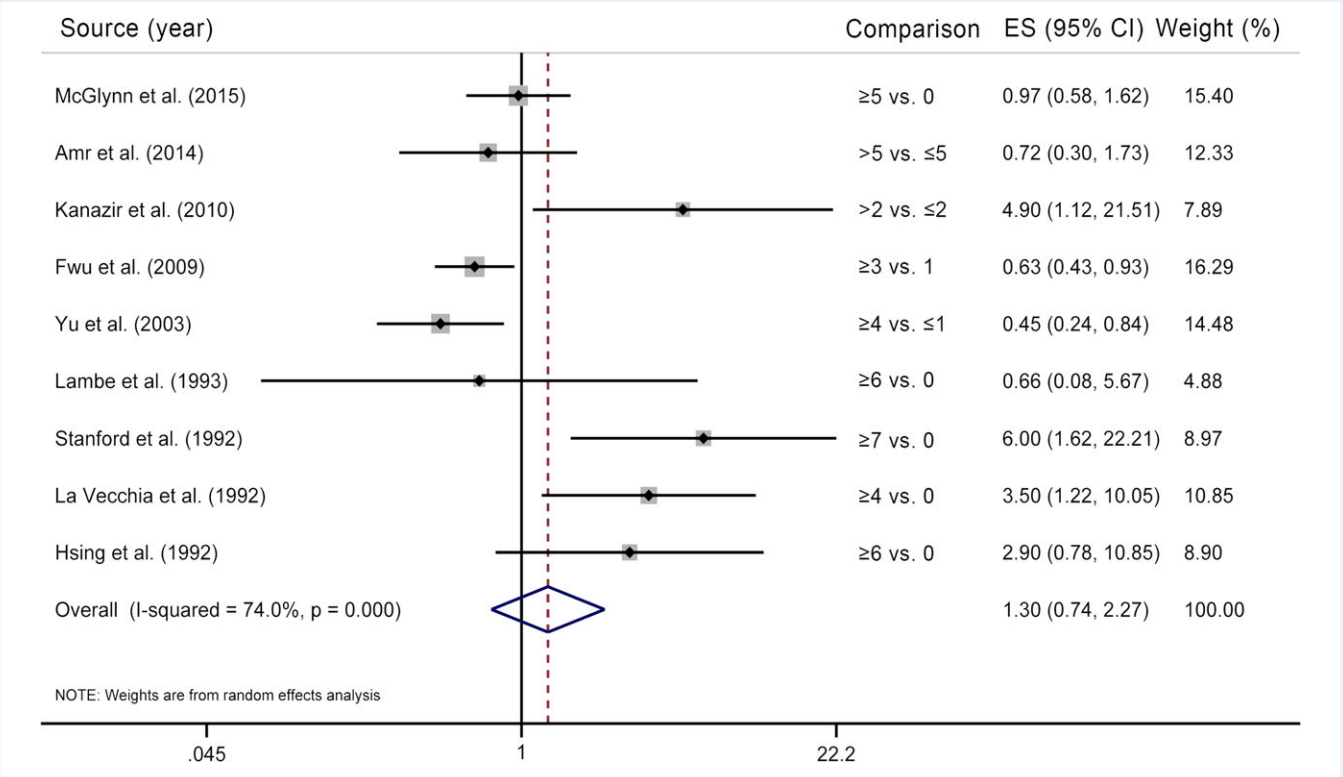

Figure 3 Highest versus lowest meta-analysis on parity and PLC risk. The squares represent the risk estimate for each individual study, with the area reflecting the weight assigned to the study. The horizontal line across each square represents the 95% CI. The diamond represents the summary risk estimate, with width representing 95% CI. ES, effect size.

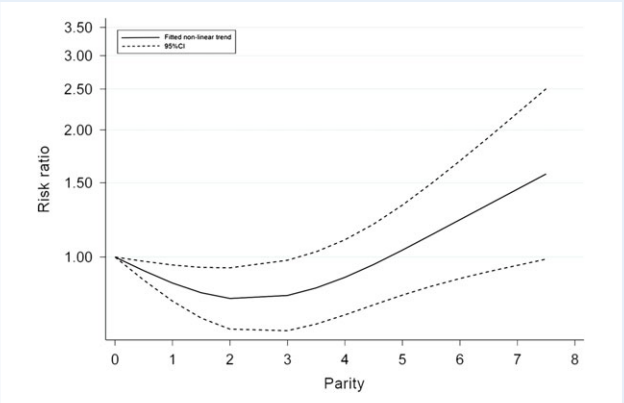

Figure 4 Non-linear dose–response analysis on parity and PLC risk.

studies contributed predominantly to the observed heterogeneity. Specifically, meta-analysis in studies with PLC cases ≥100 (Lambe *et al.*, 1993; Yu *et al.*, 2003; Fwu *et al.*, 2009; Amr *et al.*, 2014; McGlynn *et al.*, 2015) revealed an inverse association between parity and PLC risk; in contrast, meta-analysis in those with PLC cases <100 (Hsing *et al.*,1992; La Vecchia *et al.*, 1992; Stanford and Thomas, 1992; Kanazir *et al.*, 2010) revealed a positive association. We observed that the overall sample size of these studies with PLC cases <100 was also small, with the maximum sample size of only 679 subjects (Stanford and Thomas, 1992). It has been indicated that compared with larger studies, smaller studies tend to report larger risk estimates and are performed with less methodological rigor (Sterne *et al.*, 2001; Nnoaham *et al.*, 2012), which is commonly known as small study effects (Nuesch *et al.*, 2010). Four studies with <100 PLC cases (Hsing *et al.*, 1992; La Vecchia *et al.*, 1992; Stanford and Thomas, 1992; Kanazir *et al.*, 2010) were case-control studies, of which three were hospital-based (La Vecchia *et al.*, 1992; Stanford and Thomas, 1992; Kanazir *et al.*, 2010). As is well known, case-control studies, especially hospital-based ones, are more prone to selection bias and recall bias than cohort studies, and possibly provide spurious results. In addition, three of those studies (Hsing *et al.*, 1992; Stanford and Thomas, 1992; Kanazir *et al.*, 2010) with <100 PLC cases did not adjust for the common risk factors of PLC, including HBV or HCV infection, alcohol and diabetes, suggesting that their results might be biased by residual confounding. Considering the above-mentioned facts, along with the fact that a J-shaped curve was identified based on all included studies or those with ≥100 PLC cases, we concluded that parity was associated with PLC risk in a J-shaped dose–response fashion. However, it should be remembered

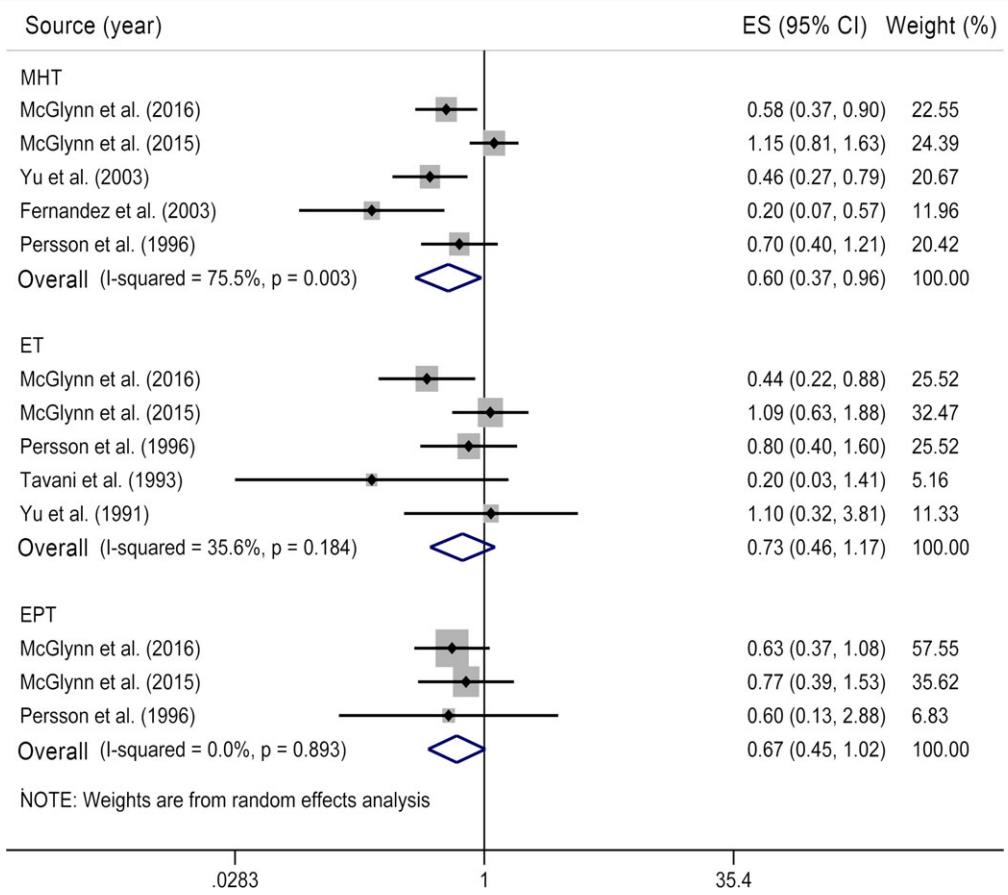

Figure 5 Meta-analyses of MHTs and PLC risk. The squares represent the risk estimate for each individual study, with the area reflecting the weight assigned to the study. The horizontal line across each square represents the 95% CI. The diamond represents the summary risk estimate, with width representing 95% CI.

that this conclusion is based on limited studies, and therefore needs to be confirmed by more large and well-designed studies.

The effect of exogenous hormone use on the PLC risk reduction has received significant attention. An early animal study found that oral contraceptive steroid was a promoter of hepatocarcinogenesis (Yager and Yager, 1980). However, a recent meta-analysis of 17 epidemiological studies suggested that there was no significant association between oral contraceptive use and PLC risk (An, 2015). To our knowledge, no meta-analysis has been conducted to investigate the association between use of MHTs and PLC risk. Therefore, the present study is the first meta-analysis addressing this association. Interestingly, our results suggested that compared with never users, ever users of MHT were at a decreased risk of PLC, whereas there was no significant difference in PLC risk between ever users and never users of ET and EPT. To interpret the above difference related to hormonal formulation is challenging. One straightforward explanation for the non-significant result on the association of ET and EPT with PLC risk is the lack of power, considering the relatively wide CIs for risk estimates and limited studies available for the association. In fact, if including a conference abstract submitted by Botrus *et al.* (2015) and then pooling corresponding risk estimates with those of the 5 included studies on ET and PLC risk, a significant result can be obtained [0.61 (95% CI 0.38, 0.99, $I^2 = 58.6\%$, $P_{heterogeneity} = 0.03$)]. An alternative biologically plausible explanation for this observation is that the inverse association between MHT and PLC risk is attributable predominantly to hormone therapies other than ET or EPT, such as progesterone therapy. Unfortunately, we cannot validate this explanation further as there seems to be no study evaluating the association of progesterone therapy with PLC risk. Taken together, the results on MHTs and PLC risk should be interpreted with caution, and more studies are needed.

The postmenopausal period is considered a crucial transition time for women's health, and good menopausal health carries substantial societal benefits (Jaspers *et al.*, 2015). Age at menopause is thought to reflect cumulative lifetime exposure to reproductive hormones (Joffe and Bromberger, 2016), and its determinants mainly include socioeconomic position, lifestyle and dietary factors, race/ethnicity, heritability and reproductive history (Meschia *et al.*, 2000; Lawlor *et al.*, 2003; Nagel *et al.*, 2005; Shobeiri and Nazari, 2014). Early age at menopause has been related to many adverse health outcomes, including increased risks of type 2 diabetes (Brand *et al.*, 2013), dementia (Coppus *et al.*, 2010), heart failure (Ebong *et al.*, 2014), venous thromboembolism (Canonico *et al.*, 2014) and depression (Georgakis *et al.*, 2016). Our study attempted to quantify the

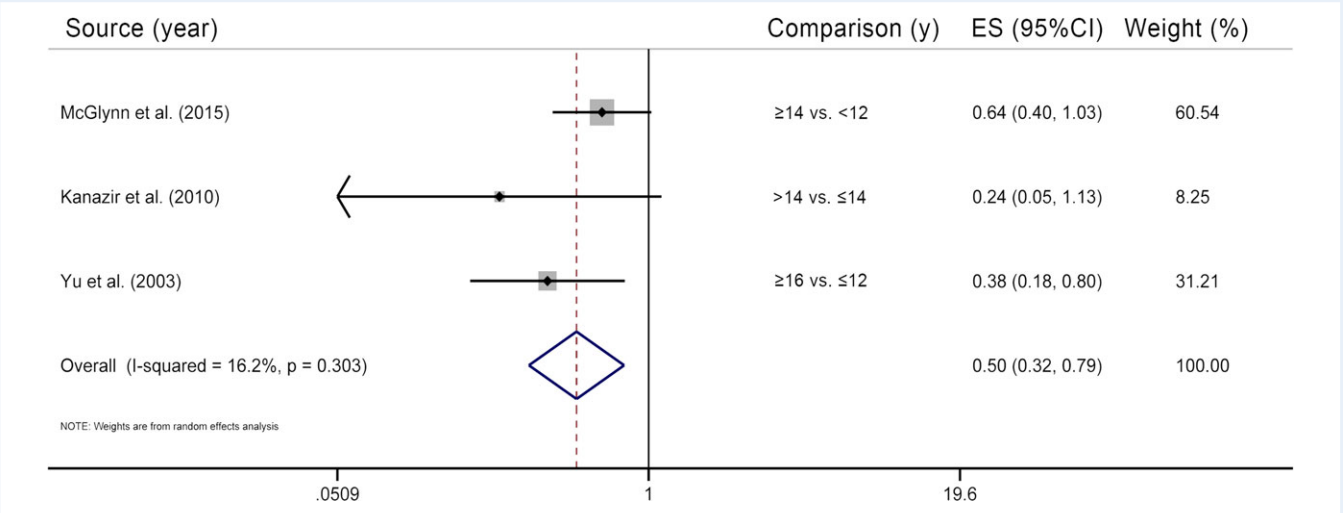

Figure 6 Oldest versus youngest meta-analysis of age at menarche and PLC risk. The squares represent the risk estimate for each individual study, with the area reflecting the weight assigned to the study. The horizontal line across each square represents the 95% CI. The diamond represents the summary risk estimate, with width representing 95% CI. y, year.

relationship between age at menopause and PLC risk; however, huge heterogeneity across studies precluded our attempt. Consequently, we could not determine the magnitude and direction of the relationship of age at menopause with PLC risk. Nevertheless, a clinical retrospective study found that early age at surgical menopause significantly increased the risk of nonalcoholic fatty liver disease among women with endometrial cancer (Matsuo *et al.*, 2016). Considering the positive association between nonalcoholic fatty liver disease and PLC risk (Zoller and Tilg, 2016), along with the protective effect of estrogen against PLC identified by basic research, we speculate that women with early age at menopause are at higher risk of PLC than those with late age at menopause. Nonetheless, this speculation needs to be confirmed by further studies.

Late age at menarche has been associated with decreased risk of ovarian cancer (Gong *et al.*, 2013) and endometrial cancer (Gong *et al.*, 2015). Similarly, in the present study, highest versus lowest meta-analysis revealed an inverse association between menarcheal age and PLC risk. Considering that age at menarche is a proxy measure of duration of exposure to endogenous estrogens, such a finding appears to be contradictory to the protective effect of estrogen on PLC risk identified by animal studies (Naugler *et al.*, 2007). In addition to age at menarche, our meta-analysis investigated other measures in relation to estrogen levels in women, including parity, MHTs and oophorectomy. We found that increasing parity and MHT use were associated with a decreased risk of PLC, and oophorectomy was associated with an increased risk of PLC; obviously, these findings are inconsistent with that in relation to age at menarche, considering that parity and oophorectomy can significantly increase and decrease serum estrogen levels, respectively. However, it should be pointed out that our finding on age at menarche and PLC risk is derived from the category analysis. The categorization of continuous variables is criticized for the inappropriate assumption of homogeneity of risk within categories, and multiple comparisons that increase the chance of false positive results (Bennette and Vickers, 2012). In fact, a null association of age at menarche with PLC risk would be observed if performing a random-effects dose–response meta-regression analysis (RR for every 1 year delay 0.97, 95% CI 0.89, 1.05), even though this analysis is based on only two studies (Yu *et al.*, 2003; McGlynn *et al.*, 2015). Moreover, age at menarche is determined by many factors, including socioeconomic status (Deardorff *et al.*, 2014; Krieger *et al.*, 2015), race/ethnicity (Deardorff *et al.*, 2014; Krieger *et al.*, 2015) and BMI (Song *et al.*, 2014). The association of socioeconomic status (Shebl *et al.*, 2012), race/ethnicity (Ha *et al.*, 2016) and BMI (Berentzen *et al.*, 2014) with PLC risk has been well documented. However, of three included studies on age at menarche and PLC risk, only one study adjusted for these potential confounders. Therefore, it is possible that what seemed to be an effect of age at menarche was really an effect of these confounders on PLC risk. Taken together, we cannot exclude the possibility that our results on menarcheal age and PLC risk have been distorted, and more studies are warranted to clarify the association between age at menarche and PLC risk.

The mechanisms of action underlying the association between hormonal exposures and PLC risk observed in our study mainly involve the protective role of sex hormones in PLC. In addition to the inhibitory effect of estrogen on liver tumorigenesis described above (Fig. 1), the protective effect of prolactin as well as progesterone on PLC risk should be also considered. A recent study in a rodent model found that prolactin could prevent liver carcinogenesis via inhibition of activation of the c-Myc oncogene (Hartwell *et al.*, 2014), which is in agreement with results of an earlier study (Yamamoto *et al.*, 1995). Unfortunately, the biological effect of natural progesterone against PLC receives little attention (Yeh *et al.*, 2013). Nonetheless, megestrol acetate, a synthetic progesterone, was found to be capable of suppressing the growth of human liver cancer cells *in vitro* and *in vivo* (Zhang and Chow, 2004).

Our study has several limitations. First, considering that most of included studies were case-control studies, and used an interview to collect the information on diseases and exposures, our results might therefore be subject to recall bias. In addition, non-differential misclassification possibly occurred in classifying the type of MHTs, and therefore would tend to bias our risk estimates toward the null. Second, we cannot determine whether the associations investigated in this study differ between women with and without common risk factors of PLC through subgroup analyses, because of inclusion of only a few studies. Third, we extracted maximally adjusted risk estimates, but the possibility of residual confounding cannot be excluded, given that our findings originate from observational studies where residual confounding always exists. Fourth, we did not assess the publication bias using the formal statistical tests, as they have insufficient power when there are limited studies ($n < 10$). Under this scenario, we cannot rule out the possibility that our pooled results are driven by publication bias. Nevertheless, all systematic reviews and meta-analyses are subject to publication bias, due to the possibility of under-reporting of negative results or failure to identify the 'grey literature' (i.e. the literature that is not published formally by commercial publishers, including conference proceedings, magazine articles, government papers, etc.) (Haddaway *et al.*, 2015). Finally, moderate or substantial heterogeneity was observed in some analyses in our study. Nonetheless, clinical and methodological heterogeneity is always a concern for all meta-analyses, particularly for meta-analysis of observational studies. Moreover, we have explained the heterogeneity observed in the analysis of parity and PLC risk.

The findings of this study have some potential implications for public health and clinical practice. Regarding public health messages, our findings on reproductive factors and PLC risk may contribute to identifying women at higher risk of developing PLC, and entering these at-risk subjects into a surveillance program for PLC at an early stage possibly improves their clinical outcomes. Our meta-analysis showed that ever use of MHT was related to a decreased risk of PLC. Similarly, ever use of EPT is found to be capable of reducing risks of colorectal and endometrial cancer (Lin *et al.*, 2012; Chlebowski *et al.*, 2016). In terms of clinical practice, these benefits seem to encourage clinicians to continue prescribing MHTs to postmenopausal women. However, it should be remembered that EPT use is known to be associated with increased risks of breast (Greiser *et al.*, 2005; Chlebowski *et al.*, 2015) and ovarian cancer (Greiser *et al.*, 2007; Pearce *et al.*, 2009). Therefore, clinicians should weigh carefully the inferred benefits of MHTs against risks involved for a particular patient before making a formal decision. Fortunately, a recent publication from the Women's Health Initiative Estrogen-Alone Trial indicates that adverse event rates of conjugated equine estrogen therapy among postmenopausal women are low and predominantly limited to current users (Jungheim and Colditz, 2011; LaCroix *et al.*, 2011), which attenuates the safety concern of MHTs to some extent.

Previous studies have examined the relationship of hormonal exposures to risks of many cancers, including lung cancer (Greiser *et al.*, 2010), esophageal cancer (Wang *et al.*, 2016), breast cancer (Pan *et al.*, 2014) and ovarian cancer (Greiser *et al.*, 2007). The current study extended this relationship into a broader field, and highlighted the association of hormonal exposures with PLC risk. Despite the contribution of our study to this field, however, there are still some critical issues that need to be addressed, including dose–response relationship between hormonal exposures and cancer risks, associations of timing of MHT use (i.e. ever/current/former use) with cancer risks, and risk-to-benefit profile of MHT use. When it comes to PLC, in addition to the above issues, further studies are warranted to investigate additional risk factors for identifying more at-risk patients and implementing better prevention measures.

Conclusions

Our study suggests a J-shaped relationship between parity and PLC risk. Late age at menarche and ever use of MHT reduce PLC risk, whereas no association of ever use of ET and EPT, age at first birth, and spontaneous or induced abortion with PLC risk is found. Women undergoing an oophorectomy have an increased risk of PLC. Our findings provide some epidemiological support for a role of hormonal exposures in the development of PLC in women. However, these findings should be interpreted with much caution owing to the limited number of studies and potential biases, and the need for further validation. Future studies will benefit from an improved design, a large sample size and better control of confounding, and should highlight the potential dose–response effects on reproductive factors, use of MHTs and PLC risk.

Supplementary Data

Supplementary data are available at http://humupd.oxfordjournals.org/.

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Authors' roles

X.D. and G.C.Z. conceived the study idea. J.H.C. and K.W. performed literature search, study selection, data extraction and quality assessment. F.B.H. performed statistical analyses. Y.L. interpreted results of statistical analyses. G.C.Z. drafted the initial manuscript. C. N. and J.P.G. made critical comment and revision for the initial manuscript. X.D. had primary responsibility for the final content. All authors reviewed and approved the final manuscript.

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Conflict of Interest

None declared.

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