Reproductive factors, exogenous hormone use and risk of hepatocellular carcinoma among US women: results from the Liver Cancer Pooling Project

K A McGlynn *,1, V V Sahasrabuddhe 1, P T Campbell 2, B I Graubard 1, J Chen 1, L M Schwartz 1, J L Petrick 1, M C Alavanja 1, G Andreotti 1, D A Boggs 3, J E Buring 4,5, A T Chan 4,6,7, N D Freedman 1, S M Gapstur 2, A R Hollenbeck 8, L Hou 9, L Y King 4,6,7, J Koshiol 1, M Linet 1, J R Palmer 3, J N Poynter 10, M Purdue 1, K Robien 11, C Schairer 1, H D Sesso 4,5, A Sigurdson 1, J Wactawski-Wende 12 and A Zeleniuch-Jacquotte 13

1Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA; 2Epidemiology Research Program, American Cancer Society, Atlanta, GA, USA; 3Slone Epidemiology Center at Boston University, Boston, MA, USA; 4Department of Medicine, Brigham and Women’s Hospital, Boston, MA, USA; 5Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA; 6Channing Division of Network Medicine, Brigham and Women’s Hospital, Boston, MA, USA; 7Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA; 8AARP, Washington DC, WA, USA; 9Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; 10Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA; 11Department of Epidemiology and Biostatistics, School of Public Health and Health Services, George Washington University, Washington DC, WA, USA; 12Department of Epidemiology and Environmental Health, University at Buffalo, Buffalo, NY, USA and 13Department of Population Health, New York University School of Medicine, New York, NY, USA

Background: Hepatocellular carcinoma (HCC) occurs less commonly among women than men in almost all regions of the world. The disparity in risk is particularly notable prior to menopause suggesting that hormonal exposures during reproductive life may be protective. Exogenous oestrogenic exposures such as oral contraceptives (OCs), however, have been reported to increase risk, suggesting that estrogens may be hepatocarcinogenic. To examine the effects of reproductive factors and exogenous hormones on risk, we conducted a prospective analysis among a large group of US women.

Methods: In the Liver Cancer Pooling Project, a consortium of US-based cohort studies, data from 799,500 women in 11 cohorts were pooled and harmonised. Cox proportional hazards regression models were used to generate hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations of reproductive factors and exogenous hormones with HCC (n = 248).

Results: Bilateral oophorectomy was associated with a significantly increased risk of HCC (HR = 2.67, 95% CI = 1.22–5.85), which did not appear to be related to a shorter duration of exposure to endogenous hormones or to menopausal hormone therapy use. There was no association between OC use and HCC (HR = 1.12, 95% CI = 0.82–1.55). Nor were there associations with parity, age at first birth, age at natural menopause, or duration of fertility.

Conclusions: The current study suggests that bilateral oophorectomy increases the risk of HCC but the explanation for the association is unclear. There was no association between OC use and HCC risk. Examination of endogenous hormone levels in relation to HCC may help to clarify the findings of the current study.

*Correspondence: Dr KA McGlynn; E-mail: mcglynnk@mail.nih.gov

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Hepatocellular carcinoma (HCC), the dominant histologic type of primary liver cancer, occurs two to three times less frequently among women than men (McGlynn and London, 2011). Women also have better survival rates and lower recurrence rates after HCC treatment than do men (Ng et al, 1997; Fukuda et al, 2007). The explanation for this gender disparity is not clear. Although some major risk factors, such as infection with hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption, and cigarette smoking are more common among men, these factors do not explain, completely, the gender differences in incidence or outcome (McGlynn and London, 2011). Such differences are not as pronounced among men and postmenopausal women (Shimizu and Ito, 2007), suggesting that hormonal factors during reproductive life may be associated with reduced risk.

Early animal experiments that examined the effects of hormones on chemically induced liver tumours suggested that estrogens promoted hepatocarcinogenesis (Yager and Yager, 1980; Cameron et al, 1981; Wanless and Medline, 1982). In contrast, other experiments have reported a tumor-enhancing effect of ovariectomy on liver cancers (Vesselinovitch et al, 1980; Nakatani et al, 2001). In addition, rodent experiments have demonstrated the ability of estrogens to protect against diethylnitrosamine-induced liver cancer due to their ability to inhibit the production of interleukin-6 (IL-6), a multifunctional cytokine (Naugler et al, 2007). Whether a similar phenomenon occurs in human liver cancer is not clear.

Findings from human observational studies in regard to hormonal exposures have been contradictory. For example, some studies have suggested higher parity increases risk (Plesko et al, 1985; La Vecchia et al, 1992; Stanford and Thomas, 1992), while others have suggested that higher parity decreases risk (Yu et al, 2003; Fwu et al, 2009; Kanazir et al, 2010; Wu et al, 2011). Similarly, an association between oral contraceptive (OC) use and liver cancer has remained uncertain. Although the International Agency for Research on Cancer (IARC) concluded in 1999 that there was sufficient evidence that OCs increased risk of HCC in the absence of viral infections (1999), a meta-analysis of the same studies later concluded that the evidence for a link was uncertain (Maheshwari et al, 2007).

Very few prior studies of reproductive factors and HCC have been conducted in the US (Yu et al, 1991; Hsing et al, 1992a, b), and all prior US studies have included fewer than 75 cases. Thus, we conducted an examination of the relationship of reproductive factors and exogenous hormone use with primary liver cancer and HCC in a large pooled study of US women.

### Materials and Methods

#### Study population

All US-based cohort studies that are members of the National Cancer Institute (NCI) Cohort Consortium (http://epi.grants.cancer.gov/Consortia/cohort.html) were invited to participate in the Liver Cancer Pooling Project. For the current analysis, which is restricted to female participants, 11 cohort studies elected to participate: NIH-AARP Diet and Health Study, Agricultural Health Study, United States Radiologic Technologists Study, Breast Cancer Detection Demonstration Project Follow-Up Study, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, Women’s Health Study, New York University Women’s Health Study, Cancer Prevention Study II, Iowa Women’s Health Study, Black Women’s Health Study, and Women’s Health Initiative (Supplementary Table 1). All studies contributed de-identified data from the entire cohort following data sharing agreements approved by the NCI and each cohort’s academic institution. Studies with stored serum samples also provided samples from a subset of the population to be used for HBV and HCV testing. For the serum subset, female controls were matched to cases on age at a 2:1 ratio.

To be considered an HCC case, cohort members had to have developed HCC documented by a cancer registry report or a medical record report. HCCs were identified by ICD-10 topography code C22 (www.cdc.gov/nchs/icoi/icd10.htm) and ICD-O-3 morphology code 8170–8175 (www.who.int/classifications/icd/adaptations/ontology/en/). All cases had to be diagnosed after the cohort participant completed and returned her initial questionnaire to the parent cohort.

Among the 11 participating US-based cohorts, 837,217 women were participants. For the current analysis, 3 women were excluded due to a prior liver cancer diagnosis, and 10,459 women were excluded due to having zero follow-up time. In addition, 27,255 women from the Women’s Health Initiative were excluded because they were randomised to the menopausal hormone therapy (MHT) study arm. After exclusions, 799,500 female members of the 11 cohorts remained for the current study. During the course of follow-up, 248 women developed HCC.

#### Exposures

Reproductive factors of primary interest to the analysis included age at menarche, ever giving birth, number of children, age at first birth, age at natural menopause, bilateral oophorectomy, hysterectomy, ever use of OCs, duration of use of OCs, ever use of MHT, recency of MHT use (never, current, and former), duration of MHT use, type of MHT use (none, estrogen-only MHT, and estrogen–progesterone combination MHT), and MHT route of administration (none, oral, and non-oral). Duration of OC use was categorised in two ways as some prior studies had hypothesised that risk of HCC is not increased prior to 5 years of use (IARC, 1999). Thus, OC use was both dichotomised (<5 and ≥5 years) and was examined in shorter intervals of years (<1, 1 to <3, 3 to <6, 6 to <8, and ≥8). For MHT use, only some cohorts had information on duration, formulation, and route of administration, thus analyses of those variables were based on fewer women than analyses of ever use and timing of use. If a woman indicated that she had undergone both a bilateral oophorectomy and a hysterectomy, she was only included in the oophorectomy group. Women were included in the hysterectomy group only if they did not report having had an oophorectomy.

#### Laboratory methods

Serum samples were analysed for markers of HBV and HCV infection. For HBV, hepatitis B surface antigen (HBsAg) was detected using the Bio-Rad GS HBsAg 3.0 enzyme immunoassay (Bio-Rad Laboratories, Redmond, WA, USA) and antibody to hepatitis B core antigen (anti-HBc) was detected using the Ortho HBc ELISA test system (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA). For HCV, antibody to HCV (anti-HCV) was detected using the Ortho HCV Version 3.0 ELISA test system (Ortho-Clinical Diagnostics, Inc.) and positive results were confirmed using the Chiron RIBA HCV 3.0 SIA (Ortho-Clinical Diagnostics, Inc.). All analyses were conducted in the Protein Expression Laboratory at the Frederick National Laboratory for Cancer Research, Frederick, MD, USA, under the direction of Dr Rachel Bagi.

#### Statistical analysis

Cox proportional hazards regression models, with follow-up time as the time metric, were used to determine hazard ratios (HRs), as approximations of relative risks, and 95% confidence intervals (CIs) for the associations between reproductive factors and HCC. Initially, parsimonious models were employed that adjusted only for age (continuous) and parent cohort study. Subsequently, more inclusive models were examined that also adjusted for: alcohol consumption (grams per day; nondrinkers, ≤1.08, >1.08–3.58, >3.58–13.54, and >13.54), BMI (<25, 25–29 and ≥30 kg m–2), diabetes (no or yes), race (white or other), smoking status (never, former, and current), education (some high school or less/high school degree or GED/some college...
or vocational training/college degree/post college education). The fully adjusted model that assessed reproductive factors was also adjusted for menopausal status (premenopausal or postmenopausal), while the models examining menopausal factors (age at menopause and MHT use) were restricted to postmenopausal women. In addition to the overall analyses, sensitivity analyses which excluded the first year, and the first 2 years after baseline of follow-up, were conducted, as was an analysis which excluded virally infected cases. The proportional hazard assumption was satisfied for analyses using Cox proportional hazards modelling. Statistical significance in all analyses was set at \( P < 0.05 \) based on two-sided tests. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Characteristics of the women in the Liver Cancer Pooling Project are displayed in Table 1. A majority of the participants were white (84.5%), married or living as married (63.5%), postmenopausal (86.7%), and were non-smokers (53.5%).

Table 2 displays the relationship between reproductive factors and HCC among all of the participants. No associations were evident with age at menarche, parity (either ever having children or number of children), or age at first birth. Similarly, there was no association with use of OCs (HR = 1.12, 95% CI = 0.82–1.55). An examination of duration of OC use also found no association with HCC, whether duration was categorised as <5 years vs \( \geq 5 \) years, or was broken down more finely.

Table 3 displays the relationship between between menopausal factors and MHT and HCC. Although there was no association with age at menopause, there was a significantly increased risk of HCC associated with bilateral oophorectomy (HR = 2.01; 95% CI = 1.12–3.61). There was no association with total fertile duration, however. The analysis of MHT use found a modestly increased risk of HCC (HR = 1.35, 95% CI = 1.01–1.81), which was more evident for use of estrogen-only MHT (HR = 1.57, 95% CI = 1.05–2.35). Dividing the ever users into current or former users did not alter the results, nor did examining the duration of use. Analysis by route of administration revealed a significantly increased risk only in association with non-oral MHT, however, the result was based on only five cases.

As women who have bilateral oophorectomies prior to menopause are likely to use MHT, particularly estrogen-only MHT, analyses were run which adjusted each variable for the other. As shown in Table 4, when the analysis of bilateral oophorectomy was adjusted for MHT use, bilateral oophorectomy remained significantly associated with risk of HCC (HR = 1.92, 95% CI = 1.04–3.53). However, when the analysis of MHT use was adjusted for bilateral oophorectomy, neither MHT use (HR = 1.15, 95% CI = 0.81–1.63 for MHT use and HR = 1.09, 95% CI = 0.63–1.88 for estrogen-only MHT use) remained significantly associated with HCC.

The examination of HBV and HCV status among a subset of the participants found, as anticipated, that HBV and HCV infections were more common among the cases than the controls. Among the 82 HCC cases tested, 31.7% \( (n = 26) \) were positive for anti-HCV and 3.7% \( (n = 3) \) were positive for HBsAg, compared to the 177 controls where 2.3% \( (n = 4) \) were anti-HCV positive and 0.6% \( (n = 1) \) were HBsAg positive. The viral results could not be incorporated into the larger analyses, as the results were only available for a small proportion of the cases and an even smaller proportion of the controls. Sensitivity analyses were conducted, however, that dropped anti-HCV(+) and HBsAg(+) cases. The results of these analyses did not differ from the analyses that included all cases (data not shown). Similarly, the analyses that dropped cases that developed in the first year of follow-up, or in the first 2 years, had very similar results as the analyses that included all follow-up time (data not shown).

DISCUSSION

In the current pooled analysis of US-based studies, bilateral oophorectomy was associated with a significantly increased risk of HCC. Although MHT use, in particular estrogen-only MHT use, appeared to be associated with risk, the association was attenuated and no longer significant once adjustment was made for bilateral

| Table 1. Characteristics of women in the Liver Cancer Pooling Project |
|-----------------------------------|
| **Total cohort (N = 799 050)** |
| **Person-years** | **N** | **%** |
| Age at entry (years) | | |
| <50 | 127 804 | 16.0 |
| 50–59 | 257 409 | 32.2 |
| 60–69 | 334 570 | 41.9 |
| ≥70 | 79 267 | 9.9 |
| Race | | |
| White | 668 163 | 84.3 |
| Black | 93 314 | 11.8 |
| Asian/Pacific Islander | 10 965 | 1.4 |
| American Indian/Alaska Native | 2029 | 0.3 |
| Other | 15 754 | 2.0 |
| Missing | 8825 | — |
| Body mass index (kg m\(^{-2}\)) | | |
| <25 | 353 321 | 45.7 |
| 25–29.9 | 250 330 | 32.3 |
| ≥30 | 169 936 | 22.0 |
| Missing | 25 463 | — |
| Education | | |
| Some high school or less | 45 498 | 5.9 |
| High school | 184 095 | 23.9 |
| Some college/vocational | 285 764 | 37.1 |
| College degree | 128 277 | 16.7 |
| Post college education | 126 506 | 16.4 |
| Missing | 28 910 | — |
| Marital status | | |
| Married/living as married | 494 230 | 63.5 |
| Not married/not living as married | 283 714 | 36.5 |
| Missing | 21 106 | — |
| Menopausal status | | |
| Premenopausal | 105 238 | 13.3 |
| Postmenopausal | 683 399 | 86.7 |
| Missing | 10 413 | — |
| Diabetes | | |
| No | 741 149 | 94.4 |
| Yes | 44 020 | 5.6 |
| Missing | 13 881 | — |
| Alcohol (grams per day) | | |
| Non-drinker | 249 628 | 33.0 |
| <1.08 | 180 449 | 24.0 |
| >1.08–3.58 | 121 278 | 16.1 |
| >3.58–13.54 | 118 449 | 15.7 |
| ≥13.54 | 84 515 | 11.2 |
| Missing | 45 731 | — |
| Cigarette smoking status | | |
| Non-smoker | 418 382 | 53.5 |
| Former smoker | 272 081 | 34.8 |
| Current smoker | 91 909 | 11.7 |
| Missing | 16 678 | — |

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The other reproductive variables were also not associated with risk of HCC.

The results on the current study in regard to bilateral oophorectomy agree with the sole prior study to examine a relationship between oophorectomy and HCC (Yu et al, 2003). That result, from a high-rate country, and the current result, are also consistent with the results of animal studies, which have reported an increased risk of liver cancer and accelerated growth of liver tumours after ovariectomy (Vesselinovitch et al, 1980; Goldfarb and Pugh, 1990; Nakatani et al, 2001). The association with oophorectomy, however, appears somewhat inconsistent with the lack of association with age at natural menopause. The current study also found no association between total duration of fertility and risk, suggesting that the increased risk with bilateral oophorectomy might be related to factors other than a decrease in estrogen levels. For example, oophorectomy has been shown to alter lipid levels among humans (Lobo, 2007) and to increase hepatic androgen receptors among rodents (Tejura et al, 1989). In addition, several studies have reported increased mortality risk after oophorectomy, though no study has specifically examined HCC (Gierach et al, 2013; Parker et al, 2013).

In prior studies, the reproductive factor most frequently examined for a relationship with liver cancer has been parity (Miller et al, 1980; Polsko et al, 1958; La Vecchia et al, 1992; Stanford and Thomas, 1992; Tzonou et al, 1992; Hsing et al, 1992b; Lambe et al, 1993; Kvale et al, 1994; Mucci et al, 2001; Yu et al, 2003; Lambe et al, 1994).
and Thomas, 1992), it was suggested that parity might only with increasing parity (Yu et al, 2001). As several of the earlier studies were from regions where HBV is the dominant risk hypothesis, however, has not been supported by more recent, later studies may be due to the relatively small (³0 years) number of earlier studies limited their study populations to women where HBV is the dominant risk factor (La Vecchia et al, 1992; Stanford and Thomas, 1992), it was suggested that parity might only increase risk among HBVþ women (Tzonou et al, 1992). This hypothesis, however, has not been supported by more recent, larger, studies from Taiwan, where HBV is the dominant risk factor. Three studies from Taiwan have reported decreased risks with increasing parity (Yu et al, 2003; Fwu et al, 2009; Kanazir et al, 2010) or null associations (Lambe et al, 1993; Kvale et al, 1994; Mucci et al, 2001). As several of the earlier studies were from regions where HBV is the dominant risk factor (La Vecchia et al, 1992; Stanford and Thomas, 1992), it was suggested that parity might only increase risk among HBVþ women (Tzonou et al, 1992). This hypothesis, however, has not been supported by more recent, larger, studies from Taiwan, where HBV is the dominant risk factor. Three studies from Taiwan have reported decreased risks with increasing parity (Yu et al, 2003; Fwu et al, 2009; Wu et al, 2011) and one of the studies (Yu et al, 2003) found no difference in the parity–HCC relationship between HBVþ and HBV– women. The reasons for the inconsistency in results between earlier and later studies may be due to the relatively small (<80) number of cases in the earlier studies (Miller et al, 1980; La Vecchia et al, 1992; Stanford and Thomas, 1992; Tzonou et al, 1992; Hsing et al, 1992b) and the examination of liver cancer, rather than HCC, as the main outcome. In addition, some studies were unable to adjust for other risk factors (Stanford and Thomas, 1992) or retrieved parity information solely from death certificates (Plesko et al, 1985). The current finding of a null association with parity is consistent with the results of the only prior US study (Hsing et al, 1992b), and with the results of studies from other low-rate HCC countries such as Canada, Sweden, and Norway (Miller et al, 1980; Lambe et al, 1993; Kvale et al, 1994). Why parity would be inversely associated with HCC in high-rate countries, but not in low-rate is unclear, but may be related to other, undetermined factors.

Age at first birth has been examined in six prior studies, of which five found no evidence of a relationship with liver cancer (Miller et al, 1980; La Vecchia et al, 1992; Stanford and Thomas, 1992; Tzonou et al, 1992; Lambe et al, 1993). The results of the current study agree with these findings. One prior study from a high-rate region, however (Wu et al, 2011), reported that older age at first birth increased risk. As that study also found that higher parity decreased risk, the age-at-first-birth finding perhaps was not surprising. In that study, the ages at first birth were higher than in the current study so it is possible that older ages at first birth (>30 years) could confer increased risk. Too few women in the current study gave birth for the first time at these ages to permit examination of that hypothesis.

Age at menarche has been reported to have no association with liver cancer risk by three studies (La Vecchia et al, 1992; Tzonou et al, 1992; Kanazir et al, 2010), while two studies reported that later age at menarche decreased risk (Mucci et al, 2001; Yu et al, 2003). Although the current study found no significant association with HCC, the risk (HR = 0.64) was lowest among women with menarche at ages 14+ years. It is thus conceivable that if there were greater numbers of women with older ages at menarche, the association might attain statistical significance. If older age at menarche is inversely related to risk, however, such a finding would argue against lifetime estrogen exposure protecting against the development of HCC.

Age at natural menopause has been examined previously in the same five studies that examined age at menarche. Three of the studies found no association (La Vecchia et al, 1992; Tzonou et al, 1992; Kanazir et al, 2010), while one small study found that later age at menopause increased risk (Mucci et al, 2001) and the other found that it decreased risk (Yu et al, 2003). The current study, in agreement with three of the prior five studies, found no association with HCC.

Prior findings suggested that use of MHT might reduce risk of HCC, as MHT has been inversely associated with fatty liver disease, liver enzyme levels and the development of diabetes in post-menopausal women (Clark et al, 2002; Kanaya et al, 2003; McKenzie et al, 2006). The current study, however, found a modest increased risk of HCC associated with MHT use (HR = 1.35), which was no longer statistically significant once adjustment was made for bilateral oophorectomy. In contrast, three prior studies of MHT reported inverse associations (Persson et al, 1996; Fernandez et al, 2003; Yu et al, 2003), while one small study from the US reported no association (Yu et al, 1991), though none of the studies reported adjustment for oophorectomy. It is conceivable that only certain MHT formulations reduce risk or that all formulations only reduce risk in a subset of women. For example, a study from Taiwan reported risk reductions only among women who were not virally infected (Yu et al, 2003). Unfortunately, information on specific MHT formulations was not available in the current study and the HBV/HCV status of most women could not be determined.

The other exogenous hormonal exposure of interest, OCs, has been more widely studied for an association with HCC than has MHT. Oral contraceptives were linked to the development of HCC in high-rate regions, however (Yu et al, 2003), found no difference in the parity–HCC relationship between HBVþ and HBV– women. The reasons for the inconsistency in results between earlier and later studies may be due to the relatively small (<80) number of cases in the earlier studies (Miller et al, 1980; La Vecchia et al, 1992; Stanford and Thomas, 1992; Tzonou et al, 1992; Hsing et al, 1992b) and the examination of liver cancer, rather than HCC, as the main outcome. In addition, some studies were unable to adjust for other risk factors (Stanford and Thomas, 1992) or retrieved parity information solely from death certificates (Plesko et al, 1985). The current finding of a null association with parity is consistent with the results of the only prior US study (Hsing et al, 1992b), and with the results of studies from other low-rate HCC countries such as Canada, Sweden, and Norway (Miller et al, 1980; Lambe et al, 1993; Kvale et al, 1994). Why parity would be inversely associated with HCC in high-rate countries, but not in low-rate is unclear, but may be related to other, undetermined factors.

Table 4. Associations of oophorectomy and MHT with hepatocellular carcinoma after adjustment of each variable for the other, the Liver Cancer Pooling Project

| Age at menopause | HR | 95% CI |
|------------------|----|-------|
| Natural menopause | <45 | 0.70  0.33, 1.49 |
|                  | 45–49 | 1.14  0.72, 1.81 |
|                  | 50–54 | 1.00  Referent |
|                  | ≥55  | 0.56  0.25, 1.24 |
| Surgical menopause | Bilateral oophorectomy | 1.92  1.04, 3.53 |
|                  | Hysterectomy | 1.25  0.78, 2.01 |
|                  | Missing | —— |
| MHT | | |
| Ever used MHT | | |
| Never | 1.00  Referent |
| Ever use | 1.15  0.81, 1.63 |
| Missing | —— |
| MHT formulation | None | 1.00  Referent |
| Estrogen only | 1.09  0.63, 1.88 |
| Combination | 0.77  0.39, 1.54 |
| Unknown | 2.13  0.87, 5.22 |
| Missing | —— |

Abbreviations: BMI = body mass index; CI = confidence interval; HR = hazard ratio; MHT = menopausal hormone therapy.

*Adjusted for age, alcohol, BMI, diabetes, race, smoking, parent cohort study, and education.
*Adjusted for age and duration of MHT.
*Also adjusted for age at menopause.

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| MHT | | |
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*Adjusted for age and duration of MHT.
*Also adjusted for age at menopause.
younger than age 50 years, whereas the majority of women in the current study were older than age 50 years. Thus, the current data argue that prior OC use is not linked to increased risk among women aged 50 years and older.

The current report is the largest study of reproductive factors and liver cancer or HCC conducted in the US. Other strengths include its wide geographic representation and its prospective design. In addition, sensitivity analyses that eliminated HCCs developing in the first years of follow-up, supported the results of the main analysis. Limitations, however, include that questions were asked in varying manners across studies and some data that would have been desirable to investigate, such as specific MHT formulations, were not able to be included. Other limitations included the inability to adjust the analysis for HBV and HCV infection status due to the limited availability of serum specimens, and the lack of information on pre-existing liver disease among the participants. In addition, only 20% of the women were younger than age 50 years at study enrolment and only 15% were non-white, so extrapolation of the findings to other groups of women should be done with caution.

The finding in rodent models that lower liver cancer risk among females may be due to oestrogenic inhibition of IL-6 production has stimulated interest in whether the same phenomenon might exist in humans (Naugler et al, 2007). Although the current study does not find a great deal of evidence to suggest that oestrogenic exposures throughout life reduce the risk of HCC in some women, the current study could not compare women known to have higher estrogen levels with women known to have lower levels.

In conclusion, the pooled analysis of data from 11 prospective US studies found the oophorectomy significantly increased the risk of HCC. Other reproductive variables, including OC use, were unrelated to risk. As the reproductive variables examined are only proxy measures of oestrogenic exposures, future studies that include serum measures of hormone levels may be able to provide further clarity on whether endogenous hormones increase risk of HCC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

KAM was involved in study conception and design, analysis and interpretation of data, and study supervision. VVS contributed to analysis and interpretation of data. PTC was responsible for acquisition of data, analysis, and interpretation of data. BIG and JLP contributed to statistical analysis and interpretation of data. JC and LMS contributed to statistical analysis. MCA, GA, DAB, JEB, ATC, NDF, SMG, ARH, LH, LYK, JK, ML, JRP, JNP, MP, KR, CS, HDS, AS, JW-W, and AZ-J were involved in acquisition of data and critical reading of the manuscript.

REFERENCES

Cameron R, Imaida K, Ito N (1981) Promotive effects of ethinyl estradiol in hepatocarcinogenesis initiated by diethylnitrosamine in male rats. *Gan* 72(2): 339–340.

Clark JM, Brancati FL, Diehl AM (2002) Nonalcoholic fatty liver disease. *Gastroenterology* 122(6): 1649–1657.

Fernandez E, Gallus S, Bosetti C, Franceschi S, Negri E, La Vecchia C (2003) Hormone replacement therapy and cancer risk: a systematic analysis from a network of case-control studies. *Int J Cancer* 105(3): 408–412.

Fukuda S, Iamoto T, Amano H, Kohashi T, Ohdan H, Tashiro H, Ashara T (2007) Clinicopathologic features of hepatocellular carcinoma patients with compensated cirrhosis surviving more than 10 years after curative hepatectomy. *World J Surg* 31(2): 345–352.

Fwu CW, Chien YC, Kirk GD, Nelson KE, You SL, Kuo HS, Feinleib M, Chen CJ (2009) Hepatitis B virus infection and hepatocellular carcinoma among parous Taiwanese women: nationwide cohort study. *J Natl Cancer Inst* 101(14): 1019–1027.

Gierach GL, Pfeiffer RM, Patel DA, Black A, Schaier C, Gill A, Brinton LA, Sherman ME (2013) Long-term overall and disease-specific mortality associated with benign gynecologic surgery performed at different ages. *Menopause* 21(6): 592–601.

Goldfarb S, Pugh TD (1990) Ovariectomy accelerates the growth of microscopic hepatocellular neoplasms in the mouse: possible association with whole body growth and fat deposition. *Cancer Res* 50(21): 6779–6782.

Henderson BE, Preston-Martin S, Edmondson HA, Peters RL, Pike MC (1983) Hepatocellular carcinoma and oral contraceptives. *Br J Cancer* 48(3): 437–440.

Hsing AW, Hoover RN, McLaughlin JK, Co-Chien HT, Wacholder S, Blot WJ, Fraumeni Jr JR (1992a) Oral contraceptives and primary liver cancer among women. *Cancer Causes Control* 3(1): 43–48.

Hsing AW, McLaughlin JK, Hoover RN, Co-Chien HT, Blot WJ, Fraumeni Jr JR (1992b) Parity and primary liver cancer among young women. *J Natl Cancer Inst* 84(14): 1118–1119.

IARC (1999) *Hormonal Contraception and Post-Menopausal Hormonal Therapy*. Vol. 72. IARC Press: Lyon, France.

Kanaya AM, Herrington D, Vittinghoff E, Lin F, Grady D, Bittner V, Cauley JA, Barrett-Connor E (2003) Glycemic effects of postmenopausal hormone therapy: the Heart and Estrogen/progestin Replacement Study. A randomized, double-blind, placebo-controlled trial. *Ann Int Med* 138(1): 1–9.

Kanazir M, Boricic I, Delic D, Tepavcovic DK, Knezevic A, Jovanovic T, Cinader B, Davis FG (1980) A study of cancer, parity and age at first livebirth. *Cancer* 46(6): 1120–1123.

Kvale G, Heuch I, Nilsson S (1994) Parity in relation to mortality and cancer incidence: a prospective study of Norwegian women. *Int J Epidemiol* 23(4): 691–699.

La Vecchia C, Negri E, Franceschi S, D’Avanzo B (1992) Reproductive factors and the risk of hepatocellular carcinoma in women. *Int J Cancer* 52(3): 351–354.

Lambe M, Trichopoulous D, Hsieh CC, Ekborg A, Pavia M (1993) Parity and hepatocellular carcinoma. A population-based study in Sweden. *Int J Cancer* 55(5): 745–747.

Lobo RA (2007) Surgical menopause and cardiovascular risks. *Menopause* 14(3 Pt 2): 562–566.

Maheshwari S, Sarraj A, Kramer J, El-Serag HB (2007) Oral contraception and the risk of hepatocellular carcinoma. *J Hepatol* 47(4): 506–513.

McGlone KA, London WT (2011) The global epidemiology of hepatocellular carcinoma: present and future. *Clin Liver Dis* 15(2): 223–243, vii-x.

McKenzie J, Fisher BM, Jaal AJ, Stanley A, Paterson K, Sattar N (2006) Effects of HRT on liver enzyme levels in women with type 2 diabetes: a randomized placebo-controlled trial. *Clin Endocrinol* 65(1): 40–44.

Miller AB, Barclay TH, Choi NW, Grace MG, Wall C, Plante M, Howe GR, Cinader B, Davis FG (1980) A study of cancer, parity and age at first pregnancy. *J Chronic Dis* 33(10): 595–605.

Miucci LA, Kuper HE, Tamini R, Lagiou P, Spanos E, Trichopoulous D (2001) Age at menarche and age at menopause in relation to hepatocellular carcinoma in women. *BJOG* 108(3): 291–294.

Nakatani T, Roy G, Fujimoto N, Ashara T, Ito A (2001) Sex hormone dependency of diethylnitrosamine-induced liver tumors in mice and chemoprevention by leuprorelin. *Ipn J Cancer Res* 92(3): 249–256.

Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M (2007) Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 317(5834): 121–124.
Ng IO, Ng M, Fan ST (1997) Better survival in women with resected hepatocellular carcinoma is not related to tumor proliferation or expression of hormone receptors. *Am J Gastroenterol* **92**(8): 1355–1358.

Parker WH, Feskanich D, Broder MS, Chang E, Shoupe D, Farquhar CM, Berek JS, Manson JE (2013) Long-term mortality associated with oophorectomy compared with ovarian conservation in the nurses’ health study. *Obstet Gynecol* **121**(4): 709–716.

Persson I, Yuen J, Bergkvist L, Schairer C (1996) Cancer incidence and mortality in women receiving estrogen and estrogen-progestin replacement therapy—long-term follow-up of a Swedish cohort. *Int J Cancer* **67**(3): 327–332.

Plesko I, Preston-Martin S, Day NE, Tzonou A, Dimitrova E, Somogyi J (1985) Parity and cancer risk in Slovakia. *Int J Cancer* **36**(5): 529–533.

Shimizu I, Ito S (2007) Protection of estrogens against the progression of chronic liver disease. *Hepatol Res* **37**(4): 239–247.

Stanford JL, Thomas DB (1992) Reproductive factors in the etiology of hepatocellular carcinoma. The WHO Collaborative Study of Neoplasia and Steroid Contraceptives. *Cancer Causes Control* **3**(1): 37–42.

Tejura S, Rodgers GR, Dunion MH, Parsons MA, Underwood JC, Ingleton PM (1989) Sex-steroid receptors in the diethylnitrosamine model of hepatocarcinogenesis: modifications by gonadal ablation and steroid replacement therapy. *J Mol Endocrinol* **3**(3): 229–237.

Tzonou A, Zavitsanos X, Hsieh CC, Trichopoulos D (1992) Liveborn children and risk of hepatocellular carcinoma. *Cancer Causes Control* **3**(2): 171–174.

Vesselinovitch SD, Itze L, Mihailovich N, Rao KV (1980) Modifying role of partial hepatectomy and gonadectomy in ethylnitrosourea-induced hepatocarcinogenesis. *Cancer Res* **40**(5): 1538–1542.

Wanless IR, Medline A (1982) Role of estrogens as promoters of hepatic neoplasia. *Lab Invest* **46**(3): 313–320.

Wu CH, Chan TF, Changhchien CC, Yang CY (2011) Parity, age at first birth, and risk of death from liver cancer: evidence from a cohort in Taiwan. *J Gastroenterol Hepatol* **26**(2): 334–339.

Yager Jr JD, Yager R (1980) Oral contraceptive steroids as promoters of hepatocarcinogenesis in female Sprague-Dawley rats. *Cancer Res* **40**(10): 3680–3685.

Yu MC, Tong MJ, Govindarajan S, Henderson BE (1991) Nonviral risk factors for hepatocellular carcinoma in a low-risk population, the non-Asians of Los Angeles County, California. *J Natl Cancer Inst* **83**(24): 1820–1826.

Yu MW, Chang HC, Chang SC, Liaw YF, Lin SM, Liu CJ, Lee SD, Lin CL, Chen PJ, Lin SC, Chen CJ (2003) Role of reproductive factors in hepatocellular carcinoma: impact on hepatitis B- and C-related risk. *Hepatology* **38**(6): 1393–1400.

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