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ORIGINAL PAPER

Fish consumption and n-3 polyunsaturated fatty acids, and risk of hepatocellular carcinoma: systematic review and meta-analysis

Min Gao · Kai Sun · Mujie Guo · Hang Gao · Kun Liu · Cuicui Yang · Sheng Li · Ning Liu

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Abstract
Purpose To investigate the association between fish consumption and n-3 polyunsaturated fatty acids (n-3 PUFA) and the risk of hepatocellular carcinoma (HCC).
Methods We identified eligible studies in MEDLINE and EMBASE up to July 2014 and the reference lists of original studies and review articles on this topic. Summary relative risks (SRR) with their 95 % confidence intervals (CI) were calculated with a random effects model.
Results Eleven studies (three cohort studies, seven retrospective case–control studies, and one nested case–control study) met eligibility criteria. Ten articles investigated fish consumption, two articles investigated n-3 PUFA, and two articles investigated alpha-linolenic acid (ALA). The current data suggest that fish consumption was associated with 35 % reduction in HCC risk (highest vs. lowest category SRRs = 0.65, 95 % CI 0.51–0.79; test for heterogeneity $p = 0.057$, $I^2 = 44.1$ %). n-3 PUFA was associated with 51 % reduction in HCC risk (highest vs. lowest category SRRs = 0.49, 95 % CI 0.19–0.79). However, no significant inverse association was found in ALA (SRRs = 0.70, 95 % CI 0.30–1.10).
Conclusion Our meta-analysis of observational studies provides evidence that fish consumption and n-3 PUFA has inverse association with the risk of HCC.

Keywords Hepatocellular carcinoma · Fish consumption · Systematic review · Meta-analysis · Relative risks

Abbreviations

HCC	Hepatocellular carcinoma
BMI	Body mass index
DM	Diabetes mellitus

Min Gao and Kai Sun have contributed equally to this study.

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PUFA Polyunsaturated fatty acids
ALA Alpha-linolenic acid
EPA Eicosapentaenoic acid
DPA Docosapentaenoic acid
DHA Docosahexaenoic acid
HBV Hepatitis B virus
HCV Hepatitis C virus

Introduction

Hepatocellular carcinoma (HCC) is a common cancer worldwide, with poor 5-year survival. An estimated 748,300 new cases and 695,900 cancer deaths occur per year, ranking it fifth among cancers for incidence and third among cancers for mortality [1]. Chronic hepatitis B (HBV) infection is the most important risk factor for HCC worldwide, especially in Asia. In Asian and African countries, more than 80 % of patients with HCC have underlying chronic HBV infection [2]. The one exception in Asia is Japan, where the prevalence of HCC has been related to chronic hepatitis C (HCV) infection [3]. In Western countries, however, chronic (HCV) infection has been determined to be present in about 60 % of patients with HCC and is the main etiologic agent leading to HCC [4, 5].

Within the context of chronic HCV and HBV infection, the presence of cirrhosis is the most important risk factor for the development of HCC. There are several modifiable risk factors in HCC, of which the most important are alcohol and tobacco [6]. Diet is also one of the most intensively studied risk factors closely related to HCC, which includes coffee and tea [7], iron [8], red and white meats [9], types of fat, selenium [10], and vitamin D [11]. However, associations with other dietary components remain unclear.

Fish plays an important role in the usual diet worldwide and is an ideal source of n-3 polyunsaturated fatty acids, which may lower cancer risk by suppressing mutations, inhibiting cellular proliferation, and inducing cell apoptosis [12, 13]. Current evidence indicated that fish consumption is inversely associated with colorectal cancer [14], lung cancer [15], and prostate cancer [16], however, not associated with breast cancer [17], gastric cancer [18], and ovarian cancer [19]. Over the past decades, so many studies have addressed the possible link between fish consumption and HCC, but the findings have been somewhat contradictory. In the present study, we therefore carried out a systematic review and meta-analysis of all available evidence of observational studies following the meta-analysis of observational studies in epidemiology guidelines [20] to clarify the association between fish consumption and risk of HCC.

Materials and methods

Data sources and searches

Two authors (M.G and K.S) independently performed a literature search using MEDLINE and EMBASE database up to 11 July 2014, and by hand searching reference lists of original studies and review articles on this topic additionally. We searched the studies with the following text words and/or medical subject heading terms: ("docosahexaenoic acid" OR "eicosapentaenoic acid" OR "docosapentaenoic acid" OR "alpha-linolenic acid" OR "polyunsaturated fatty acid" OR "omega-3 fatty acid" OR "fish") AND ("liver cancer" OR "liver neoplasms").

Study selection

We included studies that met all of the following criteria: (1) published as an original article; (2) using a case–control, cross-sectional, nested case–control, or cohort design; (3) the exposures of interest was n-3 polyunsaturated fatty acids and fish consumption; (4) the outcome of interest was HCC incidence or mortality; and (5) estimates of odds ratio (OR) or relative risk (RR) with corresponding 95 % confidence intervals (CIs) (or data to calculate them) for the highest versus non/lowest level of fish consumption were reported. Two authors (M.G and K.S) independently evaluated all of the studies retrieved from the databases. If there were multiple publications from the same study, the most relevant was selected, using the other publications to clarify methodology or characteristics of the population. We did not contact authors for the detailed information of primary studies.

Data extraction and quality assessment

Three authors (MJ.G, H.G, and K.L) independently evaluated all of the studies retrieved according to the prespecified selection criteria. Any discrepancies between reviewers were addressed by a joint reevaluation of the original article. The following information from each study was extracted using a standardized data collection form: the first author's last name, year of publication, geographic location, study design, duration of follow-up, sample size, fish consumption level, the effect estimates with 95 % CIs, and covariates adjusted in the statistical analysis.

The quality of each study was assessed independently by three reviewers (MJ.G, H.G, and K.L) using the Newcastle–Ottawa Scale (NOS). The NOS consists of three

parameters of quality: selection, comparability, and outcome (cohort studies) or exposure (case–control studies). The NOS assigns a maximum of four points for selection, a maximum of two points for comparability, and a maximum of three points for exposure or outcome. Any discrepancies between reviewers were addressed by a joint reevaluation of the original article.

Statistic analysis

We ignored the distinction between the various estimates of RR (i.e., OR, rate ratio, hazard ratio), and all measures were interpreted as RR for simplicity. As different studies might report different exposure categories (dichotomous, thirds, quarters, or fifths), we used the study-specific relative risk of the highest versus lowest category of fish consumption or n-3 PUFA exposure for the meta-analysis. We transformed the corresponding CIs into log RRs, and we calculated the corresponding variance with the use of the Greenland formula. For studies that lacked estimates, we calculated crude estimates from tabular data [21]. One study reported relative risk of HCC separately according to sex, age, and liver diseases condition [22]. We pooled these relative risks with a fixed effects model to get a summary relative risk of further meta-analysis. We used Woolf's formula to evaluate the SE of the log RRs [23]. Summary relative risk (SRR) with their corresponding 95 % CIs was combined and weighted to produce pooled RRs using a random effects model.

To investigate the sources of heterogeneity across these studies, we carried out heterogeneity tests and sensitivity analysis. In heterogeneity tests, we used the Cochran Q and I^2 statistics [24], which were used to test the differences obtained between studies due to chance. For the Q statistic, a p value of less than 0.10 was considered representative of statistically significant heterogeneity. The I^2 statistic is the proportion of total variation contributed by between-study variation. It has been suggested that I^2 values of 25, 50, and 75 % are assigned to low, moderate, and high heterogeneity, respectively [25]. We conducted sensitivity analysis to estimate the influence of each individual study on the summary results by repeating the random effects meta-analysis after omitting one study at a time. We evaluated the role of several potential sources of heterogeneity by subgroup analyses according to study design, geographical locations, study quality, and adjustments for confounding variables: HBV and HCV infections, alcohol consumption, smoking, DM, and BMI.

Funnel plots and the Egger's test were performed to test evidence of publication bias [26]. In the presence of publication bias, we used the "trim and fill" method to correct such bias [27]. Meta-analyses were carried out using STATA 12.0 (Stata Corp, College Station, TX, USA).

Result

Literature search

The detailed steps of our literature search are shown in Fig. 1. In brief, a total of 1,082 citations were obtained for review of title and abstract. Of the 1,082 citations, 1,048 were not relevant and five were duplicates. Hand searching the references of previous studies and systematic reviews identified four relevant studies. Full texts of the remaining 33 studies were retrieved for review. Three studies were excluded because exposure of interest was raw fish which might be contaminated by parasites [28–30]. One study was excluded because raw data were not applicable to calculate them [31]. Finally, 11 studies were included in the meta-analysis. (Fig. 1).

Study characteristics

Eleven articles that met our inclusion criteria in this meta-analysis were published between 1988 and 2013. There were three cohort studies [22, 32, 33] with mean follow-up time ranging from 9 to 11.4 years, seven retrospective case–control studies [12, 21, 34–38], and one nested case–control study [9]. Ten articles described the association between fish consumption and risk of HCC [9, 12, 21, 22, 32–35, 37, 38], two described the association between n-3 PUFA or ALA intake and risk [32, 36], and one reported the association between EPA, DHA, DPA intake and risk [32]. The average score for the quality assessment of included studies was 8.1 (high quality) (Table 1).

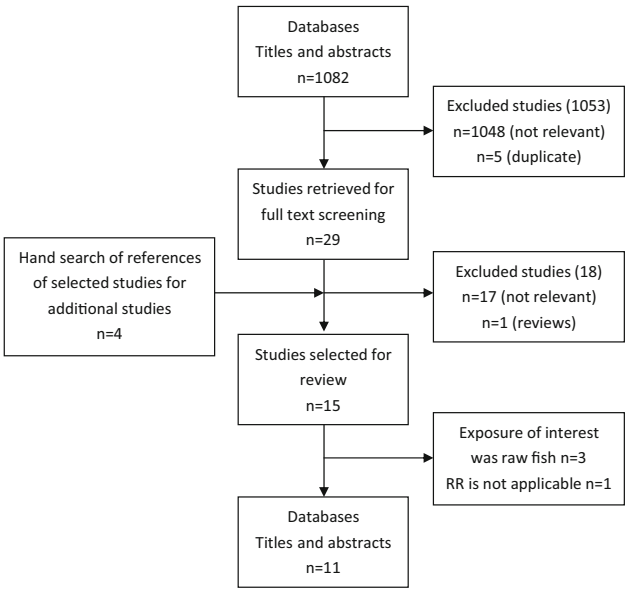

Fig. 1 Flow chart of selection of studies included in the meta-analysis

Table 1 Characteristics of 11 included studies

Author/refs.	Study published/ location	Study design	Follow-up	Cases	Non-cases	NOS	Fish consumption levels	Effect estimate (95 % CI)	Adjustments
Fedirko et al. [9]	2013/Europe	NCC	11.4	191	477,206	8	Q4 versus Q1	0.59 (0.37–0.96)	Age, sex, study center, red meat, poultry intake, smoking, physical activity, BMI, coffee, alcohol, fiber consumption
Sawada et al. [32]	2012/Japan	Cohort	11.2	398	90,296	9	Highest versus lowest n-3 PUFA ALA	0.54 (0.23–1.24) 0.51 (0.20–1.32) 0.70 (0.29–1.71)	Age, sex, area, HBV, HCV, ALT level, smoking, alcohol consumption, BMI, DM and coffee, soy food, vegetables, protein, iron intake
Daniel et al. [33]	2011/USA	Cohort	9	582	492,186	8	Q5 versus Q1	0.86 (0.65–1.13)	Age, sex, education, marital status, family history, race, BMI, smoking, physical activity, alcohol consumption, intake of meat, fruit, vegetables, and total energy
Wang et al. [34]	2011/China	PCC	–	1,116	12,395	8	≥4 servings/wk versus ≤3 servings/mo	0.72 (0.49–1.08)	Age, sex, education, physical activity, occupation, smoking, alcohol consumption, intake of meat, fruit, tea
Kanazir et al.[35]	2010/Serbia	HCC	–	40	90	7	Weekly versus rarely	0.3 (0.1–0.7)	Age, sex, area, occupation, age at menarche, menopause, parity, oral contraceptive use, breast feeding, smoking, coffee and alcohol consumption
Talamini et al. [37]	2006/Italy	HCC	–	185	412	9	Q4 versus Q1	0.83 (0.40–1.70)	Age, sex, study center, education, HBV, HCV, alcohol consumption, energy intake
Polesel et al. [7]	2007/Italy	HCC	–	185	412	9	Highest versus lowest n-3 PUFA ALA	0.48 (0.24–0.94) 0.70 (0.37–1.34)	Age, sex, study center, education, HBV, HCV, alcohol consumption, energy intake
Kurozawa et al. [22]	2004/Japan	Cohort	10	NA	110,792	7	Almost daily versus ≤1–2 servings/week	M: 0.69 (0.34–1.03)[a] F: 0.51 (0.06–0.96)[a]	Age, sex, family history, smoking, alcohol consumption, physical exercise, occupation, diet
Yu et al. [38]	2002/China	PCC	–	248	248	9	≥3 servings/ week versus <3 servings/ mo	0.32 (0.12–0.86)	Age, sex, education, occupation, HBV, HCV, smoking, alcohol consumption, diet, blood transfusion
Fernandez et al. [12]	1999/Spain, Italy	HCC	–	428	7,990	8	≥2 servings/ week versus <1 servings/ week	1.0 (0.7–1.3)	Age, sex, area, education, smoking, alcohol consumption, and BMI
La vecchia et al. [21]	1988/Italy	HCC	–	151	1,051	7	Highest versus lowest	0.75 (0.47–1.22)[b]	Age, sex

NA data not applicable, *M* male, *F* female, *PCC* population-based case–control study, *HCC* hospital-based case–control study, *NCC* nested case–control study, *Q* quartile

[a] Pooled analysis of subgroup relative risk

[b] The RR and 95 % confidence intervals were derived from raw data

Meta-analysis

Meta-analysis of ten studies in a random effects model found that fish consumption decreased the risk of HCC by 35 %, with moderate heterogeneity among studies (SRRs = 0.65, 95 % CI 0.51–0.79; test for heterogeneity $p = 0.057$, $I^2 = 44.1$ %) (Fig. 2).

In a sensitivity analysis, the overall homogeneity and effect size were calculated by removing one study at a time. We found that there were no changes in the direction of effect when any one study was excluded, supporting the stability of the inverse association between fish consumption and risk of HCC.

We subsequently conducted subgroup systematic review and meta-analysis according to study design, geographical locations, study quality, and adjustments for confounding variables: HBV and HCV infections, alcohol consumption, smoking, DM, and BMI (Table 2). In stratified analysis by geographical locations, a significant inverse association between fish consumption and risk of HCC was found for studies conducted in both Europe (SRRs = 0.68; 95 % CI 0.41–0.94; $p_{\text{heterogeneity}} = 0.025$, $I^2 = 64.2$ %) and Asia (SRRs = 0.58; 95 % CI 0.42–0.75; $p_{\text{heterogeneity}} = 0.514$, $I^2 = 0$ %). However, no significant inverse association was found in America (SRRs = 0.86; 95 % CI 0.62–1.10).

The SRRs were statistically significant in nested case–control studies (SRRs = 0.59; 95 % CI 0.29–0.88), cohort studies (SRRs = 0.73; 95 % CI 0.56–0.90; $p_{\text{heterogeneity}} = 0.451$, $I^2 = 0$ %), and population-based case–control studies (SRRs = 0.54; 95 % CI 0.15–0.93; $p_{\text{heterogeneity}} = 0.098$, $I^2 = 63.6$ %). However, no significant inverse association was found in hospital-based case–control studies (SRRs = 0.71; 95 % CI 0.35–1.06; $p_{\text{heterogeneity}} = 0.012$, $I^2 = 72.4$ %) (Table 2).

In stratified analysis by study quality, significant inverse associations were found for NOS > 7 (SRRs = 0.71; 95 % CI 0.54–0.88; $p_{\text{heterogeneity}} = 0.107$, $I^2 = 42.6$ %) and NOS 2 7 (SRRs = 0.55; 95 % CI 0.33–0.77; $p_{\text{heterogeneity}} = 0.221$, $I^2 = 31.9$ %).

Hepatitis B virus and HCV infections, alcohol consumption, smoking, DM, and BMI are important confounders for risk of HCC. When we limited the meta-analysis to studies that controlled for one of the above confounders, significant inverse associations could still be found in adjustment for HBV and HCV infections ($n = 3$, SRRs = 0.47; 95 % CI 0.20–0.74; $p_{\text{heterogeneity}} = 0.390$), alcohol consumption ($n = 6$, SRRs = 0.64; 95 % CI 0.49–0.80; $p_{\text{heterogeneity}} = 0.039$), smoking ($n = 6$, SRRs = 0.63; 95 % CI 0.47–0.80; $p_{\text{heterogeneity}} = 0.026$), and BMI ($n = 4$, SRRs = 0.78; 95 % CI 0.58–0.98; $p_{\text{heterogeneity}} = 0.179$). However, no significant inverse association was found in adjustment for DM ($n = 3$, SRRs = 0.54; 95 % CI 0.04–1.05) (Table 2).

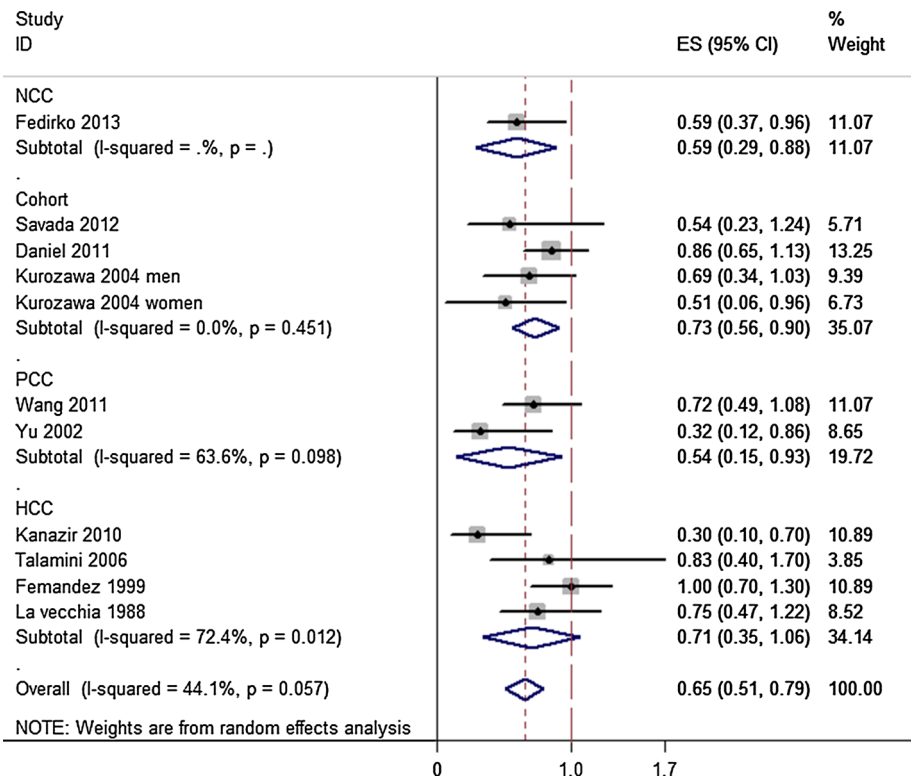

Fig. 2 Forest plot of HCC risk associated with fish consumption

Table 2 Subgroup analysis of relative risks of the association between fish consumption and hepatocellular carcinoma

Subgroup	References	Relative risk (95 % CI)	Tests for heterogeneity	
			P	I^2 (%)
Geographical region				
Europe	[9, 12, 21, 35, 37]	0.68 (0.41, 0.94)	0.025	64.2
America	[33]	0.86 (0.62, 1.10)	NA	NA
Asia	[22, 32, 34, 38]	0.58 (0.42, 0.75)	0.514	0
Study quality				
NOS > 7	[9, 12, 32–34, 37, 38]	0.71 (0.54, 0.88)	0.107	42.6
NOS ≦ 7	[21, 22, 35]	0.55 (0.33, 0.77)	0.221	31.9
Study design				
NCC	[9]	0.59 (0.29, 0.88)	NA	NA
Cohort	[22, 32, 33]	0.73 (0.56, 0.90)	0.451	0
PCC	[34, 38]	0.54 (0.15, 0.93)	0.098	63.6
HCC	[12, 21, 35, 37]	0.71 (0.35, 1.06)	0.012	72.4
Adjustment for confounders				
Adjustment for HBV and HCV	[32, 37, 38]	0.47 (0.20, 0.74)	0.390	0
No adjustment for HBV and HCV	[9, 12, 21, 22, 33–35]	0.69 (0.53, 0.85)	0.057	48.9
Adjustment for alcohol	[9, 12, 22, 32–35, 37, 38]	0.64 (0.49, 0.80)	0.039	49.1
No adjustment for alcohol	[21]	0.75 (0.37, 1.13)	NA	NA
Adjustment for smoking	[9, 12, 22, 32–35, 38]	0.63 (0.47, 0.80)	0.026	54.1
No adjustment for smoking	[21, 37]	0.77 (0.45, 1.09)	0.834	0
Adjustment for DM	[32]	0.54 (0.04, 1.05)	NA	NA
No adjustment for DM	[9, 12, 21, 22, 33–35, 37, 38]	0.66 (0.51, 0.81)	0.040	49.0
Adjustment for BMI	[9, 12, 32, 33]	0.78 (0.58, 0.98)	0.179	38.9
No adjustment for BMI	[21, 22, 34, 35, 37, 38]	0.56 (0.40, 0.72)	0.248	23.8

Two studies reported the association between n-3 PUFA and risk of HCC, n-3 PUFA was inversely associated with risk (SRRs = 0.49, 95 % CI 0.19–0.79; test for heterogeneity $p = 0.929$, $I^2 = 0$ %) (Fig. 3). Two studies reported the association between ALA and risk of HCC, ALA was not associated with risk (SRRs = 0.70, 95 % CI 0.30–1.10; test for heterogeneity $p = 0.999$, $I^2 = 0$ %) (Fig. 3). Only one study reported EPA, DPA, DHA, and risk of HCC, and hazard ratios for the highest vs lowest category were 0.55 (95 % CI 0.22–1.39) for EPA, 0.55 (95 % CI 0.21–1.42) for DPA, and 0.59 (95 % CI 0.22–1.57) for DHA in subjects who were anti-HCV or HBsAg positive.

Publication bias

Slight publication bias was observed in the literature based on the Begg's test ($p = 0.04$) (Fig. 4) and Egger's regression test ($p < 0.01$). Trim and fill analysis, however, did not change the result (SRRs = 0.69, 95 % CI 0.52–0.87), suggesting that the effect of publication bias could be negligible.

Discussion

In this meta-analysis, fish consumption may decrease the risk of HCC by as much as 35 %. Dietary intake of n-3 PUFA, but not alpha-linolenic acid (ALA), was associated with a lower risk of HCC. This is the first meta-analysis summarized the evidence to date regarding the association between fish consumption and n-3 PUFA and risk of HCC.

The inverse association between fish consumption and risk of HCC is biologically plausible. Consumption of fish provides high-quality protein, unsaturated essential fatty acids, as well as certain vitamins and minerals. Using animal models, researchers have found that supplementing the diet of tumor-bearing mice or rats with purified n-3 fatty acids has slowed the growth of HCC [39]. *Larsson* summarized current knowledge of the potential mechanisms of the anti-carcinogenic actions of n-3 fatty acids : (1) suppression of arachidonic acid-derived eicosanoid biosynthesis, (2) influence on transcription factor activity, gene expression, and signal transduction, (3) alteration of estrogen metabolism, (4) increased or decreased production of free radicals and reactive oxygen species, and (5) effect on insulin sensitivity and membrane fluidity [40].

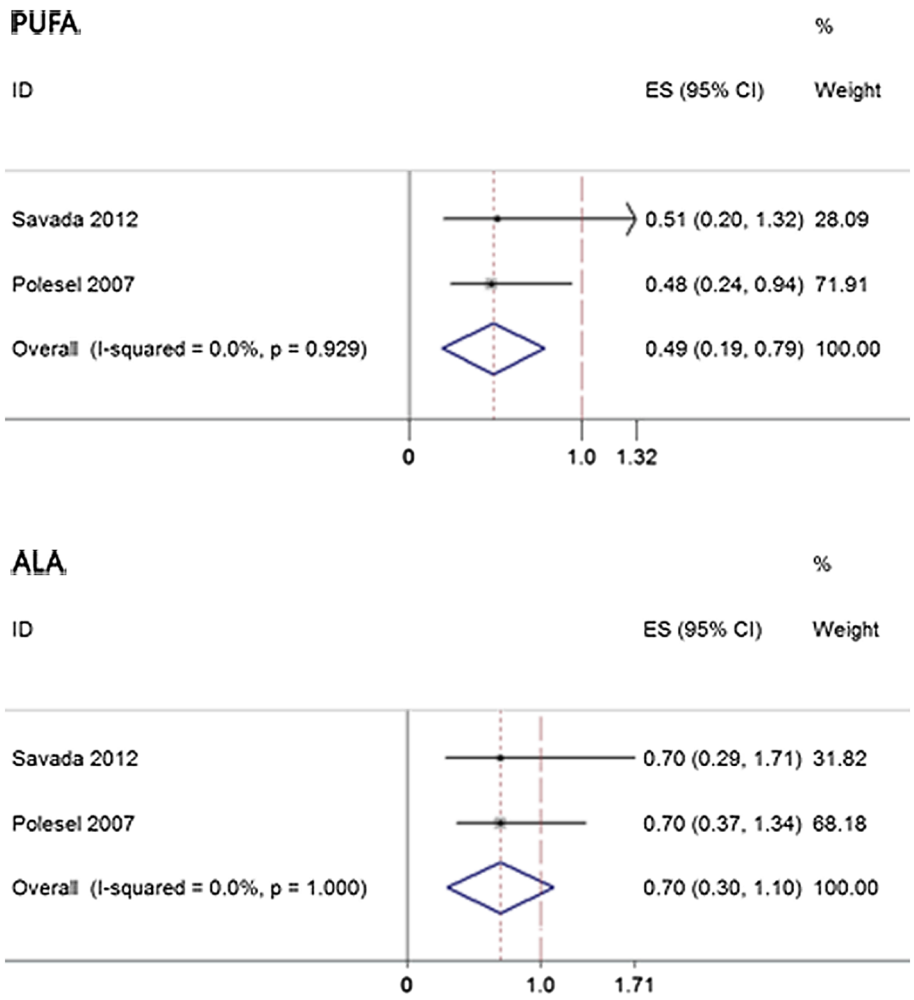

Fig. 3 Forest plot of HCC risk associated with intake of n-3 PUFA and ALA

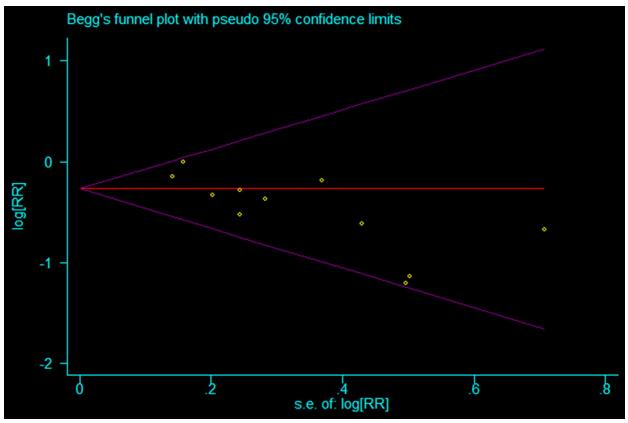

Fig. 4 Funnel plot of studies evaluating the association between fish consumption and HCC risk. Begg's test ($p = 0.04$)

There may be reasons for the discrepancies observed between included studies. First, self-reported dietary intake (especially via food frequency questionnaire) is notoriously poor and plagued by problems of random error and systematic error associated with participant characteristics. Second, the protective effect of fish consumption on HCC risk may be counterbalanced by the negative effect of contaminants. Among contaminants found in fish are methylmercury [41], polychlorinated dibenzo-*p*-dioxins [42], dibenzofurans, organochlorine residues, and other chemicals. These chemicals have high toxicity and carcinogenic potency, and a few epidemiological studies suggested that pesticides and some of these chemicals may be related to HCC [43]. Third, variation in cooking methods across study populations on these studies may have contributed to the inconsistent findings. Heterocyclic amines and polycyclic aromatic hydrocarbons formed during preparation of the fish at high temperatures may be one of the reasons. Fourth, fish is also a source of n-6 fatty acids, which can enhance growth and promotion of breast cancer, pancreatic cancer, and prostate cancer [44–46]. But there is still few experimental or clinical evidence about n-6 fatty acids intake and the risk of HCC. Fresh water fish contain lower levels of n-3 fatty acids but higher levels of n-6 fatty acids than marine fish. Most of the studies included in our meta-analysis, however, did not specify

what type of fish was consumed. Fifth, only 3 of 10 studies in this meta-analysis controlled for HBV and HCV infections. The preexisting liver disease, such as hepatitis and cirrhosis, occurs well before HCC, which may affect dietary intake of fatty acid. The protective effects of n-3 PUFA may be underestimated.

There was significant heterogeneity observed across studies, but the heterogeneity is moderate and acceptable with $I^2 = 44.1\ \%$, so we could still be able to combine studies in a meta-analysis. We analyzed this review in both fixed effects and random effects and they varied a little. The more conservative one, random effects, was chosen finally. When we tried to carry out subgroup analysis according to study design, geographical locations, study quality, and adjustments to investigate sources of heterogeneity, statistical heterogeneity was lower in Asian group and cohort studies. This suggests that study design and geographical locations might account for heterogeneity observed.

Our meta-analysis has several strengths. (1) Studies were included after a comprehensive and systematic search of the literature by using an extensive search strategy. (2) The majority of the included studies evaluated multiple confounders including HBV and HCV infections, alcohol consumption, smoking, DM, and BMI. (3) With available evidence and enlarged number of studies to date, we have enhanced statistical power to detect any associations between fish consumption and risk of HCC.

Our meta-analysis has limitations that affect interpretation of the true results. First, seven of 11 studies in this meta-analysis used case–control design, which was more susceptible to recall and selection biases than a cohort design. On the other hand, cohort studies may be affected by detection bias. Second, there is substantial heterogeneity across studies. The heterogeneity was likely due to the variation in exposure definitions, exposure ranges, and population characteristics between studies. Third, unmeasured or uncontrolled confounding inherited from original studies is a concern in this meta-analysis. Most estimate risks were derived from multivariable models, but individual studies did not adjust for potential confounding factors in a consistent way.

In summary, our meta-analysis of observational studies provides evidence that fish consumption and n-3 PUFA has inverse association with the risk of HCC. Given the small number of studies included in this meta-analysis, further prospective cohort studies with larger sample size, well controlled for confounding factors, and more accurate assessment of fish consumption and n-3 PUFA are needed to affirm the effect of fish on HCC.

Acknowledgments The authors' responsibilities were as follows—LS and LN: contributed to the study design and writing of the manuscript; GM and SK: contributed to the data search and data collection; GMJ, GH, and LK: contributed to the data extraction and quality assessment; and YCC: contributed to the data analysis.

Conflict of interest This work was supported by a grant from the International S&T Cooperation Program of China (No. 2013DFA11150) and the Plan Project of Science & Technology from Jining city (2014JNNK21&2014JNYYF03). The authors have declared that no competing interests exist.

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