Pooled analysis of association between a genetic variant in the 3'-untranslated region of Toll-like receptor 4 and cancer risk

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ABSTRACT. Many epidemiological studies have shown the association between certain genetic variations in the Toll-like receptor 4 (*TLR4*) gene (for example, rs4986790 and rs4986791) and cancer risk. However, the results from investigations into the association between rs11536889, a genetic variant in the 3'-untranslated region of *TLR4*, and cancer risk lack consensus. We performed a meta-analysis to investigate the effect of rs11536889 on cancer risk. A total of 12 relevant case-control studies were included in this analysis (6222 cases and 7948 controls). The pooled ORs with their corresponding 95%CIs were estimated. We did not detect any association between rs11536889 and overall cancer risk (P = 0.13). However, stratification analysis by cancer type revealed a borderline statistically significant increased risk in both genotype comparison (OR for the variant genotype in the dominant model = 1.17; 95%CI = 0.98-1.40; P = 0.08) and allele comparison (OR for variant allele = 1.14; 95%CI = 0.99-1.32; P = 0.07) for prostate cancer. In contrast, no statistically significant or borderline result was found for gastric cancer. These findings indicate that rs11536889 in *TLR4* might be specifically associated with prostate

cancer. However, owing to the modest and underpowered results, and the limitations of the original studies included for analysis, further prospective studies with larger sample sizes are required to confirm our findings.

Key words: Toll-like receptor 4; Single nucleotide polymorphism; Cancer risk; rs11536889; Meta-analysis

INTRODUCTION

The Toll-like receptor (TLR) family plays an important role in innate immunity (El-Omar et al., 2008). The involvement of TLRs in the mediation of the immune response suggests that increased TLR activation may result in anticancer immunity. TLR4 is one of the most investigated TLRs and is highly expressed in dendritic cells, lymphocytes, and macrophages; *TLR4*, the gene that encodes it, is on the chromosomal locus 9q32-q33 (Kutikhin, 2011). Many single nucleotide polymorphisms (SNPs) have been identified in *TLR4*, although the functional effects of most of them have not been revealed (Kutikhin, 2011). To date, many epidemiological studies investigating the association between *TLR4* genetic variants and cancer risk have been published; however, the results are conflicting (Chen et al., 2013; Zhang et al., 2013; Zhu et al., 2013; Zou et al., 2013). To the best of our knowledge, three SNPs of *TLR4*, rs4986790 (Asp299Gly), rs4986791 (Thr399Ile), and rs11536889 (3725G>C), remain the most described and studied in relation to cancer risk. These three polymorphisms have been investigated for their potential associations with the risk of different cancers such as prostate, gastric, and hepatocellular cancer. More than five meta-analyses have been published regarding the association of rs4986790 and rs4986791 with cancer risk (Lindström et al., 2010; Chen et al., 2013; Zhang et al., 2013; Zhu et al., 2013; Zou et al., 2013). It is noteworthy that in Asian populations, rs4986790 and rs4986791 are uncommon or even absent. In contrast, the prevalence of rs11536889 in Asian populations is as high as in Western populations (Cheng et al., 2007; Zhang et al., 2013). To date, the relationship between rs11536889 and cancer risk is inconclusive. Owing to the cumulating literature on the association between rs11536889 and cancer risk, we conducted an updated meta-analysis by including all eligible case-control studies to draw a relatively comprehensive conclusion.

MATERIAL AND METHODS

Study search

We searched relevant studies in the EMBASE, Web of Science, and PubMed databases from January 1, 1980 to August 1, 2014 using the following terms: “TLR4 or Toll-like receptor 4” and “polymorphism or genetic variations” and “cancer or carcinoma or neoplasm”. Among the retrieved results, we further screened “11381G>C or 3725G>C or rs11536889 or TLR4_15844” to focus on the rs11536889 SNP. We initially identified 204 papers that had investigated the association between genetic polymorphisms in *TLR4* and cancer risk. After reviewing the titles of the papers, 81 studies were excluded. The remaining 123 papers were potentially eligible. We read the abstracts; if the papers still seemed eligible, we acquired the full texts for details. Thirty-three papers with full texts were finally judged for their eligibility according to the inclusion criteria, which were as follows: 1) the papers must have been published with a full-English text (meeting abstracts were

not eligible); 2) the papers must have reported the relationship between rs11536889 and cancer risk (comparing cancer patients with cancer-free controls); 3) the papers must have been either prospective/retrospective cohort studies or retrospective case-control studies; and 4) the papers must have reported ORs with their 95%CIs, or have provided sufficient available genotyping data to estimate them. In this study, we preferred to extract adjusted ORs because, considering other confounding factors, the adjusted estimates are more likely to reflect the true effect.

After reviewing all possibly eligible papers, we finally included 12 eligible studies from 13 papers (Zheng et al., 2004; Chen et al., 2005; Cheng et al., 2007a; Wang et al., 2009; Hishida et al., 2009, 2011; Kupcinskas et al., 2011; Shi et al., 2011; Kim et al., 2012; Shui et al., 2012; He et al., 2013; Companioni et al., 2014; Li et al., 2014) in the present meta-analysis. A flow chart of the literature search is presented in Figure 1.

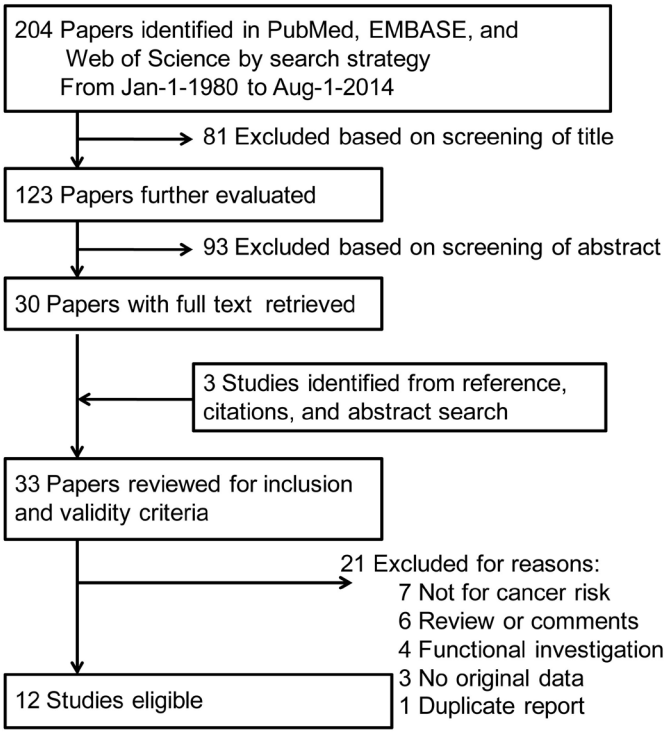

Figure 1. Flow chart for identification of studies.

This research was submitted to the Ethics Committee and Institutional Review Board of the Shanghai Cancer Center of Fudan University and was determined to be qualified for institutional review board exemption because it is a meta-analysis.

Data extraction

We extracted the following variables from every study whenever available: the first

author's name, year of publication, design type (case-control study or nested case-control from prospective cohort studies), ethnicity, numbers of cases and controls with different genotypes, and minor allele frequency (MAF). The information was collected independently by two authors (WX and XZ). If there was a discrepancy in the extracted data, a consensus was reached after discussion between the two authors. We employed the 9-star Newcastle-Ottawa Scale (http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm, accessed on August 1, 2014) to evaluate the study quality.

Statistical analysis

As described in detail previously (Wang et al., 2014), we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for reporting a systematic review about healthcare interventions (Liberati et al., 2009), and the present meta-analysis was planned, conducted, and reported in accordance with the standards of quality for reporting meta-analyses (Higgins and Green, 2011). We used the ORs and their 95%CIs to estimate the strength of association between rs11536889 and cancer risk. Two types of ORs were provided in the results: 1) GG genotype *vs* variant (GC+CC) genotype (the dominant model); and 2) G allele *vs* C allele. As no previous report had showed a significant association between rs11536889 and risk of cancer under a recessive model, we did not include the recessive model in the meta-analysis. We assessed the heterogeneity among studies using Cochran chi-square Q statistics and I-square statistics. If a P value was < 0.05 or an I-square value was > 25%, we determined that there was significant heterogeneity (Higgins et al., 2003). Use of fixed-effect (Mantel-Haenszel method) or random-effect (DerSimonian and Laird method) methods was according to heterogeneity. To show more information to readers, we provided the ORs with 95%CIs under either the fixed-effect or the random-effect models.

Assessment of the Hardy-Weinberg equilibrium (HWE) was conducted in the control population for each study using the goodness-of-fit chi-square test, and a P value less than 0.05 indicated a departure from HWE. We performed subgroup analyses by ethnicity and cancer type. As described in detail previously (Wang et al., 2014), the potential publication bias was examined visually in a funnel plot of ln[OR] against its standard error, and the degree of asymmetry was tested using the Egger test (P < 0.05 was considered to be statistically significant). We also performed sensitivity analysis (influence analysis) by omitting each study to find potential outliers. We conducted the statistical analysis using the STATA software (version 10.0. Stata Corporation, College Station, TX, USA). A two-sided P value of <0.05 was considered to be statistically significant.

RESULTS

Systematic review of studies

Twelve studies from 13 publications were included in this meta-analysis (Table 1). All the studies were of case-control design and five were nested case-control studies from prospective cohorts. Three kinds of cancer (gastric, prostate, and hepatocellular) were analyzed, with 6222 cases and 7948 controls. The detailed characteristics of the eligible studies are summarized in Table 1. The distribution of genotypes in the controls in each study was in agreement with HWE.

Among the five studies of gastric cancer (Hishida et al., 2009; Kupcinskas et al., 2011; He et al., 2013; Companioni et al., 2014; Li et al., 2014), none displayed a positive result. Among the six studies of prostate cancer (Zheng et al., 2004; Chen et al., 2005; Cheng et al., 2007a; Wang et al., 2009; Kim et al., 2012; Shui et al., 2012), two showed a statistically significant association between rs11536889 and prostate cancer risk, with ORs of 1.26 and 1.99, respectively. Only one study was on hepatocellular cancer, and it failed to show a positive outcome (Shi et al., 2011).

Table 1. Characteristics of the studies included in the meta-analysis.

First author	Country	Ethnicity	Design	Cancer type	rs11536889 genotype distribution						HWE	MAF	Quality
					Cases (N = 6222)			Controls (N = 7948)					
					GG	GC	CC	GG	GC	CC			
Chen et al. (2005)	USA	Caucasian (97%)	Nested CC	Prostate	515	167	10	513	159	15	Yes*	0.16	8
Cheng et al. (2007)	USA	Caucasian (82%)	CC	Prostate	385	105	16	401	93	12	Yes	0.14	7
Companioni et al. (2014)	Italy	Caucasian	Nested CC	Gastric	258	98	9	940	308	35	Yes	0.18	8
He et al. (2013)	China	Asian	CC	Gastric	146	73	12	343	175	21	Yes	0.26	6
Hishida et al. (2009, 2011)	Japan	Asian	CC	Gastric	312	222	49	827	635	130	Yes	0.39	7
Kim et al. (2012)	South Korea	Asian	CC	Prostate	240	NA	NA	223	NA	NA	Yes	0.29	6
Kupcinskas et al. (2011)	Germany	Caucasian	CC	Gastric	90	21	2	190	41	5	Yes	0.12	6
Li et al. (2014)	China	Asian	CC	Gastric	331	74	4	328	77	4	Yes	0.11	7
Shi et al. (2011)	China	Asian	CC	Hepatocellular	123	76	17	123	91	14	Yes	0.35	6
Shui et al. (2012)	USA	Caucasian	Nested CC	Prostate	909	292	32	897	291	27	Yes	0.17	8
Wang et al. (2009)	USA	Caucasian	Nested CC	Prostate	178	69	7	175	71	6	Yes	0.20	7
Zheng et al. (2004)	Sweden	Caucasian	Nested CC	Prostate	1047	318	15	625	141	12	Yes	0.13	8

CC = case-control; HWE = Hardy-Weinberg equilibrium; MAF = minor allele frequency; N.A. = not available. *Yes means that the genotypes of rs11536889 in controls fulfill HWE.

Results of meta-analysis

The results of the meta-analysis are presented in Table 2. In the overall population, there was no significant difference in the rs11536889 variant genotype distribution between cases and controls under the dominant model (P = 0.13; Figure 2).

Table 2. Results of meta-analysis of rs11536889 in *TLR4* and cancer risk.

Group	Number of studies	G *vs* C (Allele)					GG *vs* GC+CC (Genotype, in dominant model)				
		Random-effects		Fixed-effects		Ph; I-square	Random-effects		Fixed-effects		Ph; I-square
		OR (95%CI)	P	OR (95%CI)	P		OR (95%CI)	P	OR (95%CI)	P	
Overall	12	1.07 (0.99-1.16)	0.10	1.06 (0.99-1.14)	0.08	0.23; 21%	1.08 (0.98-1.20)	0.13	1.07 (0.99-1.16)	0.08	0.12; 34%
Caucasian	7	1.08 (0.98-1.18)	0.11	1.08 (0.98-1.18)	0.11	0.76; 0%	1.08 (0.98-1.19)	0.11	1.08 (0.98-1.19)	0.11	0.73; 0%
Asian	5	1.09 (0.90-1.32)	0.40	1.05 (0.94-1.17)	0.42	0.03; 62%	1.10 (0.85-1.43)	0.47	1.05 (0.92-1.20)	0.48	0.01; 69%
Gastric cancer	5	1.01 (0.90-1.12)	0.89	1.01 (0.90-1.12)	0.89	0.93; 0%	1.01 (0.89-1.14)	0.88	1.01 (0.89-1.14)	0.88	0.86; 0%
Prostate cancer	6	1.14 (0.99-1.32)	0.07	1.12 (1.02-1.23)	0.02	0.06; 54%	1.17 (0.98-1.40)	0.08	1.13 (1.02-1.26)	0.02	0.03; 60%

P = P value for difference in distribution; Ph = P value for heterogeneity from Q test; CI = confidence interval; OR = odds ratio.

We then performed stratification analyses by ethnicity or cancer type. When we analyzed by ethnicity, the results indicated that the effect of variant genotype or allele on cancer risk was obvious. In contrast, when stratified by cancer type, both *TLR4* variant genotype (Figure 3) and variant allele (Figure 4) consistently showed a borderline increased risk of prostate cancer (genotype comparison in dominant model: OR = 1.17, 95%CI = 0.98-1.40, P = 0.08; allele comparison: OR = 1.14, 95%CI = 0.99-1.32, P = 0.07). However, no significant or borderline difference was observed in gastric cancer or hepatocellular cancer.

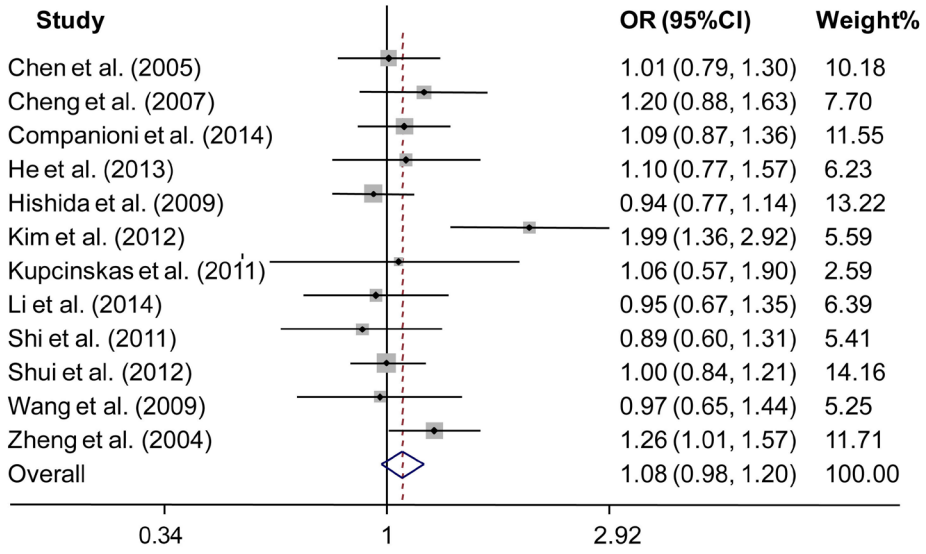

Figure 2. Forest plot of association between cancer risk and rs11536889 by genotype. The size of each square is proportional to the weight of the study. For the combined result, the length of the diamond represents the 95%CI of the summary. The genotype comparison uses the dominant model (CC+GC *vs* GG).

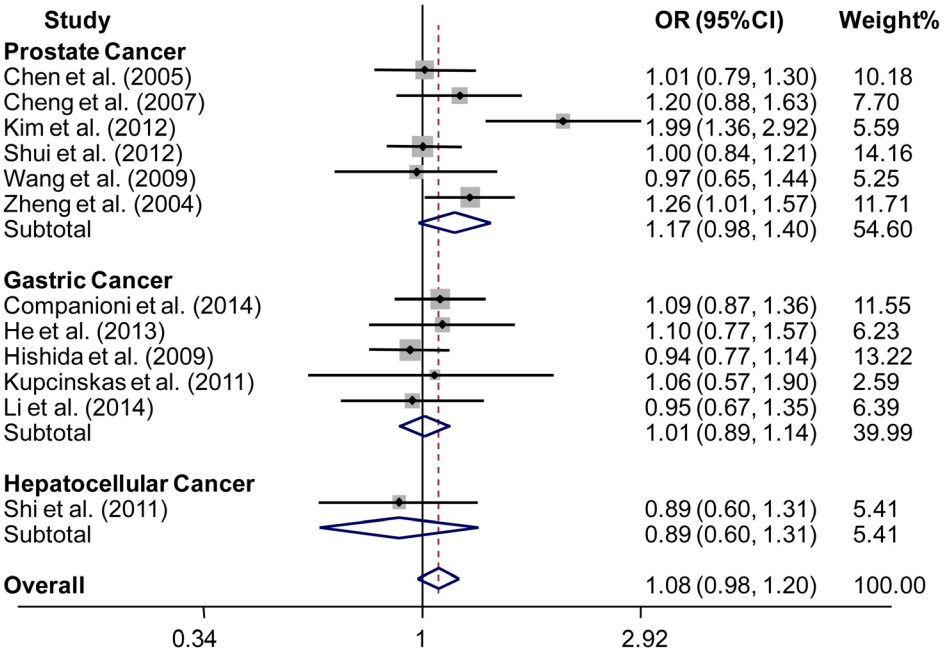

Figure 3. Forest plot of association between cancer risk and rs11536889 by genotype and stratified by cancer type.

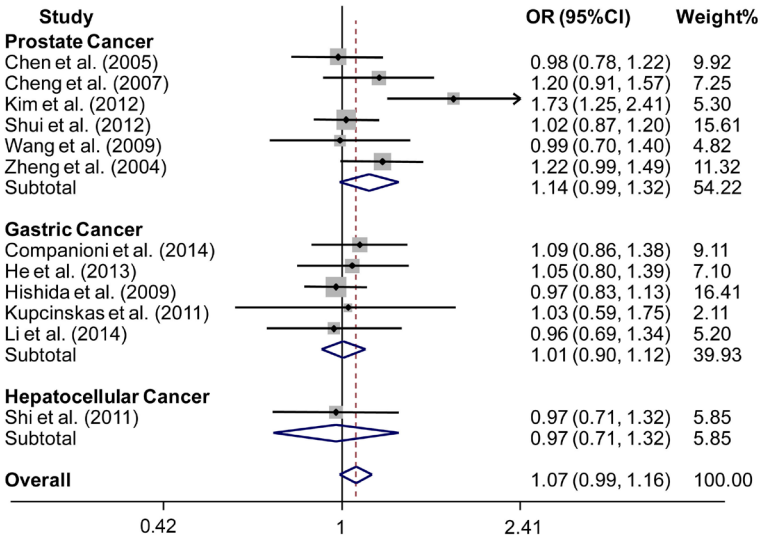

Figure 4. Forest plot of association between cancer risk and rs11536889 by allele and stratified by cancer type.

Sensitivity analyses and publication bias

We employed the leave-one-out sensitivity analysis to evaluate the robustness of the results. The results showed that no single study obviously changed the summary OR (data not shown). Inverted funnel plots (Figure 5) and Begg tests were performed to assess the publication bias, and the result suggested no obvious evidence of asymmetry (P = 0.35).

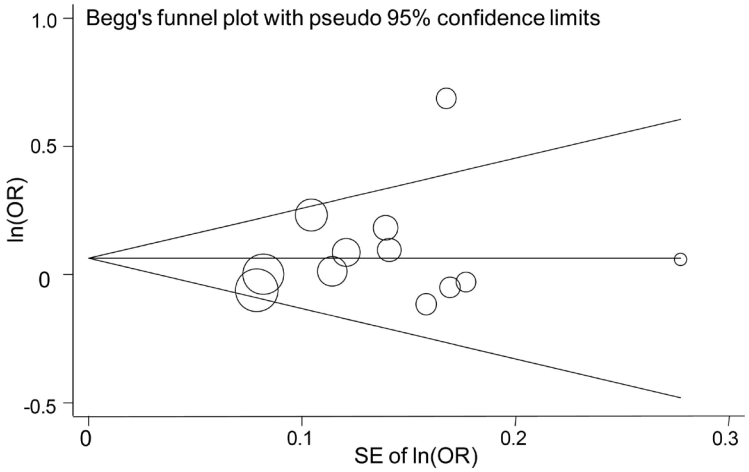

Figure 5. Funnel plot for publication bias of meta-analysis. Horizontal line and sloping lines represent summary OR and expected 95%CIs for a given standard error, respectively. The vertical axis represents ln[OR] and the horizontal axis represents the standard error of ln[OR]. The area of each circle represents contribution of a single OR from each study to the pooled OR.

DISCUSSION

The results of the current meta-analysis indicated that rs11536889 probably has no role in the risk of gastric cancer, but may play a role in the carcinogenesis process of prostate cancer. Although the P value for prostate cancer is borderline and not statistically significant, the potential association between rs11536889 and prostate cancer should not be ignored.

Three previous pooled analyses (Lindström et al., 2010; Chen et al., 2013; Zhang et al., 2013) found no positive association between rs11536889 and cancer. It should be noted that they comprised a limited number of studies: Zhang et al. (2013) included eight, Lindström et al. (2010) included three, and Chen et al. (2013) included two studies. Although our study replicated these previous outcomes to some extent, finding no significant association between rs11536889 and overall cancer, we did reveal a borderline statistically significant relationship between this SNP and prostate cancer.

The observed potential association between SNP rs11536889 and prostate cancer might be due to the linkage disequilibrium effect of other neighboring causal SNPs, or a directly biological effect of this variant. SNP rs11536889 is located in the 3'-untranslated region of *TLR4*, where a genetic change can influence the translation of TLR4 mRNA. Sato et al. (2012) showed that rs11536889 can contribute to the regulation of *TLR4* translation. They found that peripheral blood mononuclear cells from subjects carrying the C allele secreted higher levels of IL-8 than the cells from subjects with the wild-type genotype. However, previous research has suggested that serum IL-8 concentration is independent of the free/total prostate-specific antigen (PSA) ratio as a predictor of prostate cancer (Veltri et al., 1999).

The discrepancy between the different effects of rs11536889 on prostate cancer and on gastric cancer in our findings indicates that this SNP might have different biological and pathological influences in different cancer types. Furthermore, different SNPs in the same gene also have different biological consequences. For instance, previous meta-analyses have consistently indicated that *TLR4* rs4986790 (Asp299Gly) and rs4986791 (Thr399Ile) polymorphisms are risk factors for gastric cancer but not for prostate cancer. Conversely, we have shown here that rs11536889 is not associated with gastric cancer but potentially confers a risk of prostate cancer. The detailed mechanisms of these phenomena require further investigation.

Some limitations of this meta-analysis should be acknowledged. First, the subgroups may have had a relatively low power based on the low number of studies. The results from subgroup analysis should be treated with caution and the potential relationship between rs11536889 and prostate cancer risk requires further validation. Second, the controls in the included studies were recruited in different ways and were not uniformly defined, which may have induced some bias. Third, gene-gene or SNP-SNP interactions are important for the development of complex diseases including cancer because single genetic variation may only have a modest effect. However, the original genotyping data for each publication was unavailable and we could not conduct gene-gene or SNP-SNP interaction analyses in this study.

Despite these concerns, this meta-analysis provided statistical evidence that rs11536889 in *TLR4* might be specifically associated with prostate cancer. However, well-designed prospective studies with larger sample sizes are required to confirm our findings.

Conflicts of interest

The authors declare no conflict of interest.

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