Original Article
The prognostic value of T393C-SNP of *GNAS1* in patients with solid tumors

Yuping Tang[1*], Peng Song[2*], Weifeng Lu[3]

[1]Department of Orthopaedic, Nanjing Children's Hospital of Nanjing Medical University, Nanjing, China;
[2]Department of General Surgery, Drum Tower Clinical College of Nanjing Medical University, Nanjing, China;
[3]Surgical Intensive Care Unite, Nanjing Children's Hospital of Nanjing Medical University, Nanjing, China. *Equal contributors.

Abstract: Although some studies have assessed the prognostic value of T393C-SNP of *GNAS1* in patients with solid tumors, the relationship between the T393C-SNP of *GNAS1* and outcome of tumors remains unknown. We performed a meta-analysis with 15 studies, including a total of 2565 cases. Pooled hazard ratios (HRs) and 95% confidence intervals (CIs) of T393C-SNP of *GNAS1* for cancer survival were calculated. We found that individuals with the CC and CT genotypes had a statistically significant poor prognosis (HR=1.70, 95% CI=1.33-2.18, CC *vs*. TT; HR=1.41, 95% CI=1.20-1.65, CT *vs*. TT), compared with the TT genotype, respectively. In homozygote comparison (CC *vs*. TT), the risk effect was more pronounced among Caucasian patients (HR=1.70, 95% CI=1.27-2.27), with non-small cell lung cancer (HR=2.17, 95% CI=1.55-3.05), urogenital neoplasms (HR=2.57, 95% CI=1.14-4.70), publication year <2010 (HR=1.86, 95% CI=1.46-2.36) and HR estimation obtained from studies (HR=2.02, 95% CI=1.60-2.55). Subgroup analysis on No. of patients, follow time, outcomes, and quality score did not alter the significant prognostic impact of *GNAS1* T393C. Moreover, the similar results were also found in the heterozygote comparison (CT *vs*. TT). The results suggest that T393C-SNP of *GNAS1* may serve as a candidate positive marker to predict the prognosis of patients with carcinoma.

Keywords: *GNAS1*, genetic variation, cancer prognosis, meta-analysis

Introduction

During the last years, although mortality of many cancer has a marked decline due to the comprehensive treatment strategies such as surgical operation, radiotherapy and chemotherapy, the prognosis of patients still remain disappointing [1, 2]. Only some patients can derive clinical benefit from those treatments. Therefore, identifying useful prognostic predictive markers for patients with tumors, as they can help guide clinical decision regarding therapy and outcomes, is crucial and necessary.

Multiple mechanisms were implicated in tumorigenesis and prognosis, and apoptosis play a key role in development and progression of tumor. The *GNAS1* gene is located on chromosome 20q13.3. It has 13 exons and can undergo alternative splicing [3, 4]. The *GNAS1* gene encodes the G alpha subunit (Gαs) of the heterodimeric G-protein. The ubiquitously expressed Gαs is essential for coupling of multiple receptors to adenylyl cyclase and stimulation of proapoptotic processes within a cell [4-6]. The second messenger cyclic AMP (cAMP) that is generated subsequently to the activation of Gαs, seems to play a major role in this proapoptotic process. cAMP can augment or suppress extracellular regulated kinase activity [7]. The T393C-SNP of *GNAS1* affects the Gαs mRNA stability and protein expression that correlates with augmented apoptosis [8, 9]. Emerging studies identified that increased Gαs mRNA expression is a general phenomenon in individuals with the *GNAS1*393TT genotype [9]. Data from *in vitro* experiments also indicated that increased expression of Gαs enhances apoptosis [5, 6]. Hence, it is tempting to hypothesize that increased Gαs expression with concomitantly enhanced apoptosis may be prognostically favorable in patients with TT genotype.
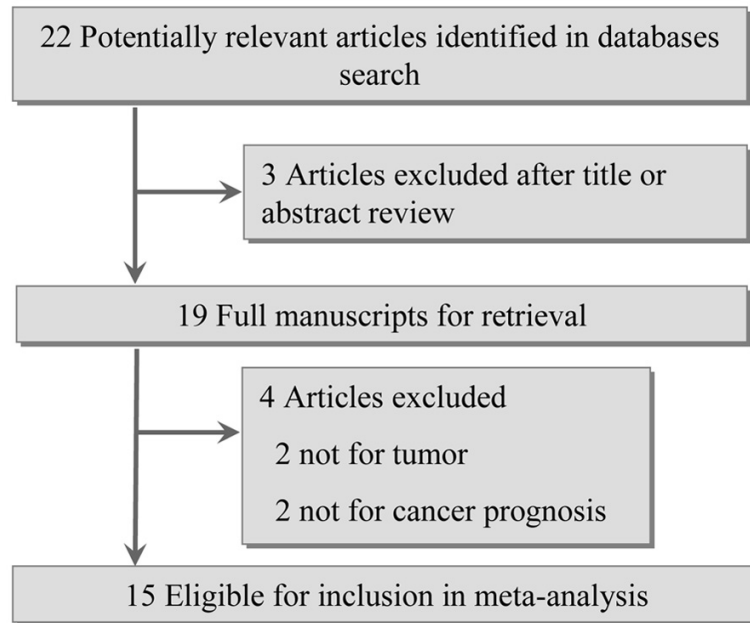
Figure 1. Studies identified with criteria for inclusion and exclusion.

However, the epidemiologic evidence regarding T393C-SNP of *GNAS1* in relation to cancer prognosis remains controversial and has not been quantitatively evaluated in a meta-analysis. Thus, we conducted a comprehensive systematic literature review and meta-analysis to assess the association between the SNP *GNAS1* T393C and cancer survival.

Material and methods

Search strategy

We used PubMed to search relevant studies that estimated the SNP *GNAS1* T393C for prognosis of tumor patients. The deadline of search was in July, 2015. References cited in the identified studies were also searched manually to obtain other suitable articles. The key words were the search string [(*GNAS1* T393C) AND (cancer OR tumor OR neoplasm)].

Data extraction

Articles were recruited in this meta-analysis if they satisfied the following criteria: (1) assessing the association between survival and the SNP *GNAS1* T393C among tumor patients; (2) providing available information estimating of HRs and 95% CIs; (3) being published in English language. Two investigators decided the eligibil-

ity of relevant reports independently and there was no disagreement after authors' discussion. We recorded the most relevant data comprising year of publication, the first author's name, cancer types, the No. of patients, laboratory method, follow time and HR resource.

Quality assessment

Study quality was scored by two investigators using the predefined form independently. Because of no generally acknowledged criterion for assessing the quality of prognostic studies, the present form in this meta-analysis was obtained from Hayes et al. [10] and McShane et al. [11] (Supplementary Table 1). Briefly, the following items were scored: (1) Does the study report exclusion and inclusion criteria? (2) Are the characteristics of patients and tumor sufficiently depicted? (3) Is the study data retrospective or prospective? (4) Is the study endpoint defined (5) Are the methods applied to detect marker expression was detailedly described? (6) Does the study present how many patients were not available for statistical analysis or were lost to follow-up? (7) Was the follow-up time of subjects provided? Articles obtained eight score were considered to be the optimal quality, while a zero score shown the inferior quality.

Statistical analysis

Pooling HRs was used to calculate the impact of SNP *GNAS1* T393C on prognosis of cancer patients. HR>1 revealed a worse prognosis, and would be deemed to be a statistically difference. Obtaining the HR estimate and 95% CI directly from the paper is the most reliable approach. If a study only provided the survival information in the form of Kaplan-Meier curve and excluded the HR and 95% CI, the HR digitizer software Engauge 4.0 and software GetData Graph Digitizer 2.24 were used to extract and digitize the survival data. Briefly, we first used GetData Graph Digitizer to open captured Kaplan-Meier curve which was obtained

Table 1. Main characeristic and results of eligible studies

Characteristics	Country	Ethnicity	Cancer types	No. of patients	Laboratory method	Outcomes	HR estimation	Follow time (months)	Quality score
Gong 2014	China	Asian	Non-small cell lung cancer	94	RFLP	OS	Survival curve	<60	<6
Uzunoglu 2013	Germany	Caucasian	Non-small cell lung cancer	163	RFLP	OS	HR	>60	≥6
Xie 2012	China	Asian	Non-small cell lung cancer	131	RFLP	OS	HR	<60	<6
Hindy 2011	Germany	Caucasian	Glioblastoma multiforme	162	RFLP	OS	Survival curve	<60	<6
Vashist 2011	Germany	Caucasian	Esophageal cancer	190	RFLP	OS	Survival curve	>60	<6
Alakus 2009	Germany	Caucasian	Gastric cancer	122	TaqMan	OS	Survival curve	>60	<6
Lehnerdt 2008	Germany	Caucasian	Laryngeal squamous cell carcinoma	157	RFLP	OS	HR	>60	<6
Lehnerdt 2008	Germany	Caucasian	Head and neck cancer, squamous cell carcinoma	202	RFLP	OS	HR	>60	≥6
Kaderi 2007	Sweden	Caucasian	Chronic lymphocytic leukemia	279	RFLP	OS	Survival curve	>60	<6
Schmitz 2007	Japan	Asian	Intrahepatic cholangiocarcinoma	87	RFLP	OS	Survival curve	>60	≥6
Otterbach 2006	Germany	Caucasian	Breast cancer	279	RFLP	OS	HR	>60	<6
Frey 2006	Germany	Caucasian	Chronic lymphocytic leukemia	144	RFLP	OS	HR	>60	≥6
Frey 2006	Germany	Caucasian	Renal cell carcinoma	150	RFLP	DFS	Survival curve	>60	≥6
Frey 2005	Germany	Caucasian	Colorectal Cancer	151	RFLP	OS	HR	>60	<6
Frey 2005	Germany	Caucasian	Bladder Cancer	254	RFLP	DFS	HR	>60	≥6
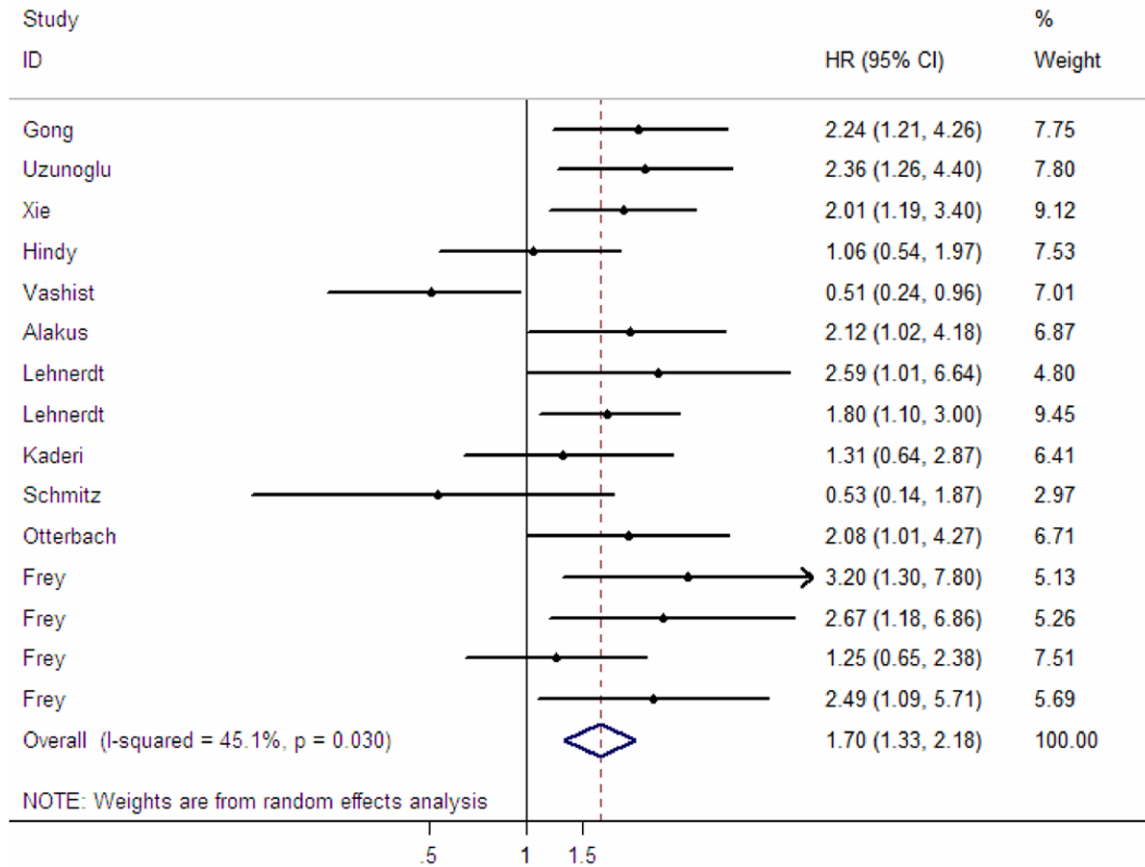
Figure 2. Forrest plots of studies evaluating hazard ratios of *GNAS1* T393C CC genotype as compared with TT genotype among patients with cancer. The squares and horizontal lines correspond to the study-specific HR and 95% CI. The area of the squares reflects the study-specific weight (inverse of the variance). The diamond represents the pooled HR and 95% CI.

from the included study, then set the scale (coordinate system) and extract the survival data manually, and last digitize the HR and 95% CI in Engauge Digitizer. The heterogeneity among studies was estimated with the *Q*-test and I^2 statistics. Fixed effect model was used if observed *Q*-test *P*>0.10 and I^2<50%. Otherwise, the random-effect model was applied. Subgroup analyses by stratifying on ethnicities, cancer types, follow time, HR resource, Publication year, number of patients and quality score were conducted to explore the resource of heterogeneity. The sensitivity analysis was conducted by removing each study in sequence to assess the stability of the present study. In addition, the publication bias was accessed by egger's test and begg's funnel plots [12]. All analyses were completed with STATA software (version 10.1; StataCorp, College Station, TX, USA).

Results

Description of studies

Figure 1 illustrated the flow diagram of the literature search and study selection. We identified 22 articles in the electronic databases. And when excluding non-related studies or lack of data on the association between *GNAS1* T393C and cancer survival, 15 publications met the inclusion criteria for the present analysis. A total of 2565 cancer patients were included in this analysis. The sample sizes ranged from 87 to 279. Of the 15 included studies, three reported on non-small cell lung cancer, five reported on digestive system neoplasms, two reported urogenital neoplasms, two reported chronic lymphocytic leukemia, and one each glioblastoma multiforme, head and neck cancer, squamous cell carcinoma and breast can-

Table 2. Stratification analyses of T393C-SNP of *GNAS1* association with prognostic risk of tumor patients

Variables	Reports, n	CC vs TT			CT vs TT		
		HR (95% CI)	I², %	P for heterogeneity	HR (95% CI)	I², %	P for heterogeneity
Total	15	1.7 (1.33-2.18)	45.1	0.03	1.41 (1.20-1.65)	0.00	0.789
Ethnicity							
Asian	3	1.69 (0.92-3.08)	50.3	0.134	1.50 (1.17-1.93)	44.1	0.167
Caucasian	12	1.70 (1.27-2.27)	48.1	0.031	1.34 (1.09-1.66)	0.00	0.899
Cancer types							
Non-small cell lung cancer	3	2.17 (1.55-3.05)	0.00	0.923	1.53 (1.21-1.94)	0.00	0.644
Digestive system neoplasms	5	1.17 (0.62-2.22)	67.9	0.014	1.02 (0.71-1.47)	0.00	0.526
Chronic lymphocytic leukemia	2	1.98 (0.83-4.73)	55.4	0.134	1.44 (0.81-2.59)	0.00	0.488
Urogenital neoplasms	2	2.57 (1.41-4.70)	0.00	0.91	1.80 (0.98-3.33)	0.00	0.519
Others	3	1.59 (1.10-2.32)	12.1	0.32	1.44 (1.00-2.06)	0.00	0.793
No. of patients							
>200	4	1.84 (1.32-2.56)	0.00	0.710	1.50 (1.08-2.09)	0.00	0.675
<200	11	1.64 (1.17-2.31)	58.0	0.008	1.38 (1.15-1.66)	0.00	0.639
Follow time (months)							
>60	12	1.70 (1.25-2.31)	50.7	0.022	1.29 (1.05-1.59)	0.00	0.731
<60	3	1.72 (1.11-2.65)	36.8	0.206	1.58 (1.24-2.03)	0.00	0.864
Outcomes							
OS	13	1.61 (1.23-2.12)	49.0	0.024	1.38 (1.17-1.63)	0.00	0.742
DFS	2	2.57 (1.41-4.70)	0.00	0.910	1.80 (0.98-3.33)	0.00	0.519
Quality score							
≥6	6	2.10 (1.49-2.96)	18.4	0.294	1.33 (1.00-1.77)	0.00	0.521
<6	9	1.51 (1.09-2.10)	51.8	0.035	1.44 (1.19-1.75)	0.00	0.734
Publication year							
>2010	5	1.45 (0.84-2.48)	73.6	0.004	1.47 (1.18-1.83)	0.00	0.690
<2010	10	1.86 (1.46-2.36)	1.50	0.425	1.34 (1.06-1.69)	0.00	0.633
HR estimation							
HR	8	2.02 (1.60-2.55)	0.00	0.771	1.50 (1.20-1.87)	0.00	0.777
Survival curve	7	1.31 (0.81-2.10)	63.6	0.010	1.31 (1.04-1.65)	0.00	0.559

Table 3. Meta-regression analysis of T393C-SNP of GNAS1 association with prognostic risk of tumor patients

Variable	Coefficient	Standard error	P value	95% CI
HR estimation	-0.881	0.389	0.041	-1.892, -0.013
No. of patients	-0.428	0.459	0.379	-1.602, 0.594
Follow time	-0.559	0.398	0.190	-1.693, 0.618
Sample size	-0.781	0.427	0.127	-1.691, 0.327

cer. Descriptive data from studies included in our meta-analysis were shown in **Table 1**.

Qualitative assessment

The scores of study quality assessed by the above quality assessment scale (Supplementary Table 1) ranged from 4 to 7 (with a mean of 5.3). Studies were categorised as high quality if the score was 7 points or more, as medium quality if the score was 4-6 points and as low quality if the score was <3.

Quantitative synthesis

Overall, individuals with the CC and CT genotypes had a statistically significant poor prognosis (HR=1.70, 95% CI=1.33-2.18, $P_{heterogeneity}$=0.03 for CC *vs.* TT; HR=1.41 95% CI=1.20-1.65, $P_{heterogeneity}$=0.789 for CT *vs.* TT), compared with the TT genotype, respectively (**Figure 2**). The main results of meta-analysis are showed in **Table 2**. Furthermore, the associations between the *GNAS1* T393C and survival of cancer patients was evaluated by
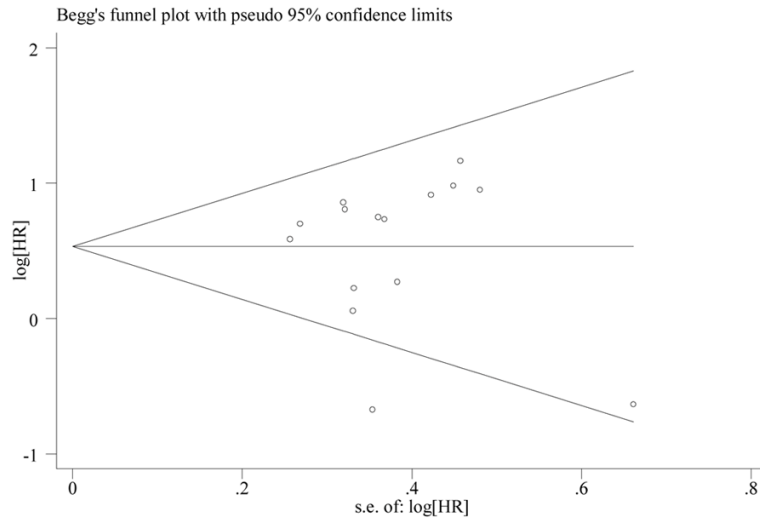
Figure 3. Funnel plot of *GNAS1* T393C and overall survival among patients with cancer. Each point represents a separate study for the indicated association. Log [HR], natural logarithm of odds ratio. Horizontal line means effect size.

stratified analysis of ethnicity, cancer types, No. of patients, follow time, outcomes, quality score, publication year, HR estimation. As the presented in **Table 2**, patients carrying CC/CT genotypes had a lower survival rate than those with the TT genotype. In homozygote comparison (CC *vs.* TT), the risk effect was more pronounced among Caucasian patients (HR=1.70, 95% CI=1.27-2.27), with non-small cell lung cancer (HR=2.17, 95% CI=1.55-3.05), urogenital neoplasms (HR=2.57, 95% CI=1.14-4.70), publication year <2010 (HR =1.86, 95% CI= 1.46-2.36) and HR estimation obtained from studies (HR=2.02, 95% CI=1.60-2.55). Subgroup analysis on No. of patients, follow time, outcomes, and quality score did not alter the significant prognostic impact of *GNAS1* T393C. Moreover, the similar results were also found in the heterozygote comparison (CT *vs.* TT), as indicated in **Table 2**.

Meta-regression

Meta-regression analysis indicated that HR estimation but not the No. of patients, follow time and sample size were significant sources of heterogeneity (**Table 3**). The estimated between-study variance (τ^2) was reduced from 0.102 to 0.086.

Publication bias

As shown in **Figure 3**, the shapes of the funnel plots did not reveal any evidence of obvious

asymmetry. Then, the Egger's test was adopted to provide statistical evidence of funnel plot symmetry, and no significant bias was detected (*t*= 0.58, *P*=0.468).

Discussion

This meta-analysis, including 2565 subjects from 15 published studies, explored the association between a potentially functional polymorphism, *GNAS1* T393C, within the *GNAS1* and cancer prognosis. We found the evidence that the variant genotypes of the *GNAS1* T393C were associated with a significant determinant of poor survival. This association was existed among Europeans, but not Asians, a possible reflection of differences in genetic background and gene-environment interactions in the etiology. However, there was no reported study in the African populations. Additionally, the risk effect of this SNP was more pronounced among Caucasian patients, with non-small cell lung cancer and urogenital neoplasms. Therefore, additional studies are warranted to further validate possible ethnic differences in the effect of this functional SNP on cancer risk.

Our finding is biologically plausible. As a vital parameter in cellular signaling Gαs is ubiquitously found [13]. Mutations in Gαs itself, but also mutations in the binding pocket of G protein-coupled receptors, can affect their downstream function [14]. Different diseases are described with gain-of-function and loss-of-function mutations such as McCune-Albright-Syndrome, a disease which combines *café-au-lait* spots, polyostotic fibrous dysplasia and endocrinopathies [15], fibrous dysplasia itself [16] or endocrinological diseases [17]. Because of the widespread occurrence of G-protein-coupled receptors mutations the gene could in principle be involved in a large number of different tumor types. And indeed, it was shown that *GNAS1* mutation could be found in different neoplasia throughout the whole body ranging from pituitary gland tumors [18], over pancreatic intraductal papillary mucinous neoplasm

[19, 20], villous adenomas of the colorectum [21] to colorectal carcinoma [22].

In this meta-analysis, we gathered a very heterogeneous group of reports, consisting of some different types of methodology, types of cancer and patient selection criteria. In spite of mentioned above, we still deem it was appropriate to pool them, as we considered that the biological functions of the SNP *GNAS1* T393C may be out of tumor type interference. However, the effect size of SNP *GNAS1* T393C in predicting prognosis of patients with cancers is undoubtedly associated with clinical pathological factors. The interactions can be adjusted in multivariate analyses. Nevertheless, for pooled analysis multivariate analysis is not applicable, because the variation of covariates between studies and outcomes calculated are based on located data in that study.

Additionally, some limitations of this meta-analysis should be acknowledged. First, many studies only provided the results without showing detailed calculation methods or the raw data. To do this, the investigators of all the published studies were encouraged to share their raw data. Second, which is better for clinical application, a single SNP or a panel of SNPs? Recently, researchers have considered using a panel of SNPs to replace a single SNP, and to add the prediction ability [23]. Third, in the meta-analysis some studies did not report HRs and CIs, but only Kaplan-Meier curves and log-rank tests. We used the software to digitize and extract the data, which might cause some imprecision. In the stratified analysis, we found the significant association in the HR estimation obtained from studies but not survival curve (**Table 2**). This highlights the importance of a uniform reporting of study outcomes and follow-up time. For routine clinical application in the future, the above-mentioned problems should be solved.

In spite of these, our meta-analysis also had several advantages. We used uniform criteria for identifying relevant studies and abstracting pertinent information. A strength of this meta-analysis was that the number of total subjects (2565) was substantial, which significantly increased the statistical power of the analysis. Moreover, we summarized risk estimates from epidemiologic studies that comprised substantial numbers of cancer case patients and were adjusted for numerous potential confounding variables, yielding precise and valid risk estimates for the association between *GNAS1* T393C and cancer prognosis. Furthermore, no evidence of publication bias was detected, which indicated the whole pooled results might be credible.

In summary, this study, showing a quantified synthesis of the related articles, identified that significant association between the *GNAS1* T393C CC and CT genotypes and poor outcome in patients with carcinoma. Before it can be applied in the routine clinical surveillance of cancer, more clinical researches should be conducted in further studies.

Disclosure of conflict of interest

None.

Address correspondence to: Weifeng Lu, Nanjing Children's Hospital of Nanjing Medical University, 72 Guangzhou Road, Nanjing 210008, Jiangsu, China. E-mail: NJWeifeng_lu@163.com

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Nagini S. Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention. World J Gastrointest Oncol 2012; 4: 156-169.

[3] Weinstein LS, Chen M and Liu J. Gs(alpha) mutations and imprinting defects in human disease. Ann N Y Acad Sci 2002; 968: 173-197.

[4] Weinstein LS, Liu J, Sakamoto A, Xie T and Chen M. Minireview: GNAS: normal and abnormal functions. Endocrinology 2004; 145: 5459-5464.

[5] Yang X, Lee FY Sr and Wand GS. Increased expression of Gs(alpha) enhances activation of the adenylyl cyclase signal transduction cascade. Mol Endocrinol 1997; 11: 1053-1061.

[6] Daaka Y, Luttrell LM and Lefkowitz RJ. Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. Nature 1997; 390: 88-91.

[7] Dumaz N, Light Y and Marais R. Cyclic AMP blocks cell growth through Raf-1-dependent and Raf-1-independent mechanisms. Mol Cell Biol 2002; 22: 3717-3728.

[8] Frey UH, Alakus H, Wohlschlaeger J, Schmitz KJ, Winde G, van Calker HG, Jockel KH, Siffert W and Schmid KW. GNAS1 T393C polymorphism and survival in patients with sporadic

colorectal cancer. Clin Cancer Res 2005; 11: 5071-5077.

[9] Frey UH, Eisenhardt A, Lummen G, Rubben H, Jockel KH, Schmid KW and Siffert W. The T393C polymorphism of the G alpha s gene (GNAS1) is a novel prognostic marker in bladder cancer. Cancer Epidemiol Biomarkers Prev 2005; 14: 871-877.

[10] Hayes DF, Bast RC, Desch CE, Fritsche H Jr, Kemeny NE, Jessup JM, Locker GY, Macdonald JS, Mennel RG, Norton L, Ravdin P, Taube S and Winn RJ. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996; 88: 1456-1466.

[11] McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M and Clark GM. REporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer 2005; 93: 387-391.

[12] Egger M, Davey Smith G, Schneider M and Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629-634.

[13] Bastepe M. The GNAS Locus: Quintessential Complex Gene Encoding Gsalpha, XLalphas, and other Imprinted Transcripts. Curr Genomics 2007; 8: 398-414.

[14] Hajjhussein H, Gardner LA, Fujii N, Anderson NM and Bahouth SW. The hydrophobic amino acid cluster at the cytoplasmic end of transmembrane helix III modulates the coupling of the beta(1)-adrenergic receptor to G(s). J Recept Signal Transduct Res 2013; 33: 79-88.

[15] Narumi S, Matsuo K, Ishii T, Tanahashi Y and Hasegawa T. Quantitative and sensitive detection of GNAS mutations causing mccune-albright syndrome with next generation sequencing. PLoS One 2013; 8: e60525.

[16] Shi RR, Li XF, Zhang R, Chen Y and Li TJ. GNAS mutational analysis in differentiating fibrous dysplasia and ossifying fibroma of the jaw. Mod Pathol 2013; 26: 1023-1031.

[17] Izzi B, de Zegher F, Francois I, del Favero J, Goossens D, Wittevrongel C, Thys C, Van Geet C and Freson K. No evidence for GNAS copy number variants in patients with features of Albright's hereditary osteodystrophy and abnormal platelet Gs activity. J Hum Genet 2012; 57: 277-279.

[18] Mantovani G, Lania AG and Spada A. GNAS imprinting and pituitary tumors. Mol Cell Endocrinol 2010; 326: 15-18.

[19] Furukawa T, Kuboki Y, Tanji E, Yoshida S, Hatori T, Yamamoto M, Shibata N, Shimizu K, Kamatani N and Shiratori K. Whole-exome sequencing uncovers frequent GNAS mutations in intraductal papillary mucinous neoplasms of the pancreas. Sci Rep 2011; 1: 161.

[20] Kanda M, Knight S, Topazian M, Syngal S, Farrell J, Lee J, Kamel I, Lennon AM, Borges M, Young A, Fujiwara S, Seike J, Eshleman J, Hruban RH, Canto MI and Goggins M. Mutant GNAS detected in duodenal collections of secretin-stimulated pancreatic juice indicates the presence or emergence of pancreatic cysts. Gut 2013; 62: 1024-1033.

[21] Yamada M, Sekine S, Ogawa R, Taniguchi H, Kushima R, Tsuda H and Kanai Y. Frequent activating GNAS mutations in villous adenoma of the colorectum. J Pathol 2012; 228: 113-118.

[22] Idziaszczyk S, Wilson CH, Smith CG, Adams DJ and Cheadle JP. Analysis of the frequency of GNAS codon 201 mutations in advanced colorectal cancer. Cancer Genet Cytogenet 2010; 202: 67-69.

[23] Ma G, Gu D, Lv C, Chu H, Xu Z, Tong N, Wang M, Tang C, Xu Y, Zhang Z, Wang B and Chen J. Genetic variant in 8q24 is associated with prognosis for gastric cancer in a Chinese population. J Gastroenterol Hepatol 2015; 30: 689-95.

Supplementary Table 1. Criteria for quality assessment

Criterium	Points
1. Is the population under study defined with in-and exclusion criteria?	1
2. Were patient data prospectively collected?	1
3. Are the main prognostic patient and tumour characteristics presented?[1]	1
4. Is the method used for determination of protein expression specified?	2
4.1. Criteria for immuhistochemistry/FISH	
Is the immunohistochemical staining protocol specified?[2]	1
Were stainings evaluated by >1 observer?	1
4.2. Criteria for mutational analysis	
Is the PCR protocol specified?[3]	1
Is the SSCP and/or sequencing protocol specified?	1
4.3. Criteria for Southern Blot	
Are the restriction enzymes used specified?	1
Is the hybridization methods specified?[4]	1
4.4. Criteria for EGF binding assay	
Are positive and negative controls specified?	1
Is the assay protocol specified?[5]	1
4.5. Criteria for RT-PCR	
Is the RNA isolation method and cDNA synthesis specified?	1
Is the PCR protocol specified?[3]	1
4.6. Criteria for enzyme immunoassay	
Is the antibody used specified?	1
Are control samples and a cut-off value for positive expression specified?	1
5. Is the study endpoint defined?	1
6. Is the time of follow up specified?	1
7. Is loss during analysis or follow up described?	1
	Max. 8 points

1) At least four of the following characteristics: age at diagnosis, FIGO stage, tumour type, differentiation grade and residual tumour after primary surgery: 2) At least four of the following criteria: antigen retrieval, primary antibody, dilution, detection method, cut-off value for positive expression: 3) At least the primers used and the annealing temperature or number of cycles: 4) At least internal controls and probes used: 5) At least four of the following criteria: label, incubation time, filter size, separation method (BSA/Tris-sucrose), cut-off value for positive expression.