RESEARCH ARTICLE

B-cell Lymphoma 2 rs17757541 C>G Polymorphism was Associated with an Increased Risk of Gastric Cardiac Adenocarcinoma in a Chinese Population

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Abstract

Aim: Apoptosis has been considered as a fundamental component in cancer pathogenesis, and related genetic factors might play an important role in gastric cardiac adenocarcinoma (GCA) genesis. Methods: We conducted a hospital based case–control study to evaluate the genetic effects of functional single nucleotide polymorphisms (SNPs): *BCL2* rs17757541 C>G, *BCL2* rs12454712 T>C, *FAS* rs2234767 G>A, *FASL*/*FASLG* rs763110 C>T, *ERBB2* rs1136201 A>G and *VEGFR2*/*KDR* rs11941492 C>T on the development of GCA. A total of 243 GCA cases and 476 controls were recruited for the study and genotypes were determined using a custom-by-design 48-Plex SNPscan™ Kit. Results: The *BCL2* rs17757541 C>G polymorphism was associated with increased risk of GCA. However, there was no significant associations with the other five SNPs. Stratified analyses indicated a significantly increased risk of GCA associated with the *BCL2* rs17757541 C>G polymorphism among males, older patients and those with a history of smoking or drinking. Conclusion: These findings indicated that the functional polymorphism *BCL2* rs17757541 C>G might contribute to GCA susceptibility. However, our results were limited by small sample size. Future larger studies are required to confirm our current findings.

Keywords: *BCL2* - polymorphisms - gastric cardiac adenocarcinoma - molecular epidemiology

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Introduction

Apoptosis, also called programmed cell death, is a major control mechanism by which cells die if DNA damage is not repaired (Lowe et al., 2000). Apoptosis has been considered as a fundamental component in cancer pathogenesis (Alenzi et al., 2010), key genes related to apoptosis include B-cell lymphoma 2 (*BCL2*) (Kroemer et al., 1997), *FAS*, *FAS* ligand (*FASL*)/*FASLG* (Nagata et al., 1999), erythroblastic leukemia viral oncogene homolog 2 (ERBB2) (Yu et al., 1998), vascular endothelial growth factor receptor 2 (*VEGFR2*)/KDR (Kasahara et al., 2000) and other genes (Nagata et al., 1997; Elmore et al., 2007). *BCL2* is initially identified as an antiapoptotic regulatory protein (Cory et al., 2002). However, it also serves as an inhibitor of proliferation (Zinkel et al., 2006). The *FASL-FAS* system mainly forms the death-inducing signaling complex (Holler et al., 2003). *ERBB2* (HER2/Neu) is a member of the ERBB family of transmembrane receptor tyrosine kinases associated with apoptosis, plays an important role in cancers (Yarden et al., 2001). VEGFR2/KDR can bind to vascular endothelial growth factor (VEGF) and result endothelial cell proliferation, migration, apoptosis inhibition and vascular structures maturation (Giatromanolaki et al., 2007).

Considering the biological and pathologic significance of *BCL2*, *FAS*, *FASL*/*FASL*G, *ERBB2* and VEGFR2/KDR, it is possible that functional genetic variations in these genes may contribute to the development of cancers such as the gastric cardiac adenocarcinoma (GCA). The objective of this investigation was to evaluate the association between functional polymorphisms *BCL2* rs17757541 C>G, *BCL2* rs12454712 T>C, *FAS* rs2234767 G>A, *FASL*/*FASLG* rs763110 C>T, *ERBB2* rs1136201 A>G and VEGFR2/KDR rs11941492 C>T variants and GCA susceptibility in a hospital-based case-control study. For this purpose we here performed genotyping analyses for these six single nucleotide polymorphisms (SNPs) using a smaple of 243 GCA cases and 476 controls in a Chinese population.

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Materials and Methods

Ethical approval of the study protocol

This hospital-based case-control study was approved by the Review Board of Jiangsu University (Zhenjiang, China). We have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals. All subjects provided written informed consent to be included in the study.

Study subjects

Two-hundred and forty three subjects with gastric cardiac cancer were consecutively recruited from the Affiliated People's Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Zhenjiang, China) between October 2008 and July 2010. All cases of gastric cardiac cancer were diagnosed as GCA by pathologic means. The exclusion criteria were patients who previously had: cancer; any metastasized cancer; radiotherapy or chemotherapy. Among 476 controls, 380 controls were patients without cancer frequency-matched to the cases with regard to age (±5 years) and sex recruited from the two hospitals mentioned above during the same time period. Another 96 controls were recruited from hospitals in Changzhou city (which is a neighbouring city of Zhenjiang) as previous described (Cheng et al., 2012).

Most of the controls were admitted to the hospitals for the treatment of trauma.

Each subject was personally questioned by trained interviewers using a pre-tested questionnaire to obtain information on demographic data (e.g., age, sex) and related risk factors (including tobacco smoking and alcohol consumption). After the interview, 2-mL samples of venous blood were collected from each subject.

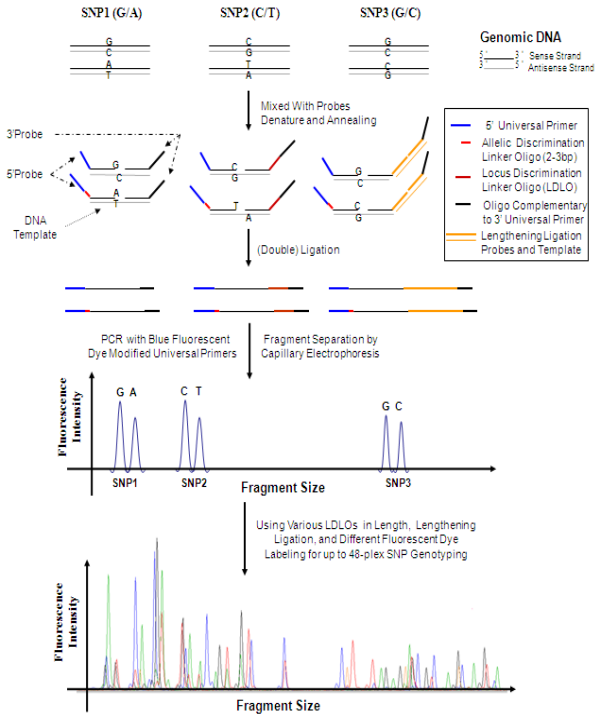

Figure 1. Illustration for 48-Plex SNP Genotyping Using SNPscanTM Kit

Individuals who smoked one cigarette per day for >1 year were defined as "smokers". Subjects who consumed ≥3 alcoholic drinks a week for >6 months were considered to be "alcohol drinkers".

Isolation of DNA and genotyping by a custom-by-design 48-Plex SNPscanTM Kit

Blood samples were collected from patients using Vacutainers and transferred to tubes lined with ethylenediamine tetra-acetic acid (EDTA). Genomic DNA was isolated from whole blood with the QIAamp DNA Blood Mini Kit (Qiagen, Germany). Sample DNA (10 ng) were amplified by PCR according to the manufacturer's recommendations. The SNP genotyping work was performed using a custom-by-design 48-Plex SNPscanTM Kit (Genesky Biotechnologies Inc., Shanghai, China) as previously described (Sun et al., 2013; Wei et al., 2013; Yin et al., 2013; Zheng et al., 2013) (Figure 1). This kit was developed according to patented SNP genotyping technology by Genesky Biotechnologies Inc., which was based on double ligation and multiplex fluorescence PCR. For quality control, repeated analyses were done for 4% of randomly selected samples with high DNA quality.

Statistical Analyses

Differences in the distributions of demographic characteristics, selected variables, and genotypes of the *BCL2* rs17757541 C>G, *BCL2* rs12454712 T>C, *FAS* rs2234767 G>A, *FASL*/*FASLG* rs763110 C>T, *ERBB2* rs1136201 A>G and VEGFR2/KDR rs11941492 C>T variants between the cases and controls were evaluated using the χ^2 test.

The associations between *BCL2* rs17757541 C>G, *BCL2* rs12454712 T>C, *FAS* rs2234767 G>A, *FASL*/*FASLG* rs763110 C>T, *ERBB2* rs1136201 A>G and VEGFR2/KDR rs11941492 C>T genotypes and risk of GCA were estimated by computing the ORs and their 95% CIs using logistic regression analyses for crude ORs and adjusted ORs when adjusting for age, sex, smoking

Table 1. Distribution of Selected Demographic Variables and Risk Factors in Gastric Cardiac Adenocarcinoma (GCA) Cases and Controls

Variable	Cases (n=243)		Controls (n=476)		P^a
	n	%	n	%	
Age (years)					0.923
< 65	126	51.9	245	51.5	
≥ 65	117	48.1	231	48.5	
Age (years) mean ± SD	64.90 (±8.65)		64.76 (±7.46)		0.832
Sex					0.197
Male	159	65.4	288	60.5	
Female	84	34.6	188	39.5	
Tobacco use					**0.004**
Never	144	59.3	333	70	
Ever	99	40.7	143	30	
Alcohol use					0.217
Never	167	68.7	348	73.1	
Ever	76	31.3	128	26.9	

[a]Two-sided χ^2 test and student t test; Bold values are statistically significant (P <0.05)

Table 2. Primary Information for Six Genotyped SNPs

Genotyped SNPs	Chr	Regulome DB Score[a]	TFBS[b]	Location	MAF[c] for Chinese in database	MAF in our controls (n = 476)	P value for HWE[d] test in our controls	% Genotyping value
BCL2: rs17757541 C>G	18	6	–	Intron3	0.134	0.146	0.726	97.36
BCL2: rs12454712 T>C	18	No Data	–	Intron3	0.429	0.453	0.404	97.22
FAS: rs2234767 G>A	10	4	Y	5'-Flanking	0.267	0.321	0.284	97.5
FASL/FASLG: rs763110 C>T	1	4	Y	5'-Flanking	0.271	0.234	0.155	97.5
ERBB2: rs1136201 A>G	17	No Data	–	nonsynon_exon17	0.13	0.129	0.179	96.94
VEGFR2/KDR: rs11941492 C>T	4	No Data	–	Intron8	0.329	0.319	0.478	97.5

[a]http://www.regulomedb.org/; [b]TFBS: Transcription Factor Binding Site (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm); [c]MAF: minor allele frequency; [d]HWE: Hardy–Weinberg equilibrium

Table 3. Logistic Regression Analyses of Associations Between Six Genotyped SNPs and Risk of GCA

Genotype	Cases (n=243)		Controls (n=476)		Crude OR (95%CI)	*P*	Adjusted OR (95%CI)[a]	*P*
	n	%	n	%				
BCL2: rs17757541 C>G								
CC	153	65.11	338	72.69	1		1	
CG	76	32.34	118	25.38	**1.42 (1.01-2.01)**	**0.046**	1.39 (0.98-1.98)	0.063
GG	6	2.55	9	1.94	1.47 (0.52-4.21)	0.47	1.45 (0.50-4.18)	0.493
CG+GG	82	34.89	127	27.31	**1.43 (1.02-2.00)**	**0.039**	1.40 (0.99-1.96)	0.054
CC+CG	229	97.45	456	98.06	1		1	
GG	6	2.55	9	1.94	1.33 (0.47-3.78)	0.595	1.32 (0.46-3.78)	0.608
BCL2: rs12454712 T>C								
TT	64	27.47	144	30.9	1		1	
TC	117	50.21	222	47.64	1.19 (0.82-1.72)	0.367	1.17 (0.81-1.70)	0.41
CC	52	22.32	100	21.46	1.17 (0.75-1.83)	0.49	1.18 (0.75-1.85)	0.471
TC+CC	169	72.53	322	69.1	1.18 (0.83-1.67)	0.35	1.17 (0.83-1.67)	0.373
TT+TC	181	77.68	366	78.54	1		1	
CC	52	22.32	100	21.46	1.05 (0.72-1.54)	0.795	1.07 (0.73-1.57)	0.73
FAS: rs2234767 G>A								
GG	112	47.66	220	47.21	1		1	
GA	100	42.55	193	41.42	1.02 (0.73-1.42)	0.917	1.01 (0.72-1.41)	0.952
AA	23	9.79	53	11.37	0.85 (0.50-1.46)	0.562	0.87 (0.50-1.50)	0.611
GA+AA	123	52.34	246	52.79	0.98 (0.72-1.34)	0.91	0.98 (0.71-1.35)	0.902
GG+GA	212	90.21	413	88.63	1		1	
AA	23	9.79	53	11.37	0.85 (0.50-1.42)	0.524	0.86 (0.51-1.46)	0.582
FASL/FASLG: rs763110 C>T								
CC	130	55.32	268	57.51	1		1	
CT	88	37.45	178	38.2	1.02 (0.73-1.42)	0.91	1.00 (0.72-1.40)	0.999
TT	17	7.23	20	4.29	1.75 (0.89-3.46)	0.106	1.79 (0.90-3.55)	0.098
CT+TT	105	44.68	198	42.49	1.09 (0.80-1.50)	0.58	1.08 (0.78-1.48)	0.646
CC+CT	218	92.77	446	95.71	1		1	
TT	17	7.23	20	4.29	1.74 (0.89-3.39)	0.104	1.79 (0.91-3.50)	0.092
ERBB2: rs1136201 A>G								
AA	185	79.74	356	76.56	1		1	
AG	45	19.4	98	21.08	0.88 (0.60-1.31)	0.539	0.85 (0.57-1.27)	0.419
GG	2	0.86	11	2.37	0.35 (0.08-1.60)	0.175	0.33 (0.07-1.52)	0.156
AG+GG	47	20.26	109	23.44	0.83 (0.56-1.22)	0.343	0.79 (0.54-1.18)	0.249
AA+AG	230	99.14	454	97.63	1		1	
GG	2	0.86	11	2.37	0.36 (0.08-1.63)	0.185	0.34 (0.07-1.57)	0.168
VEGFR2/KDR: rs11941492 C>T								
CC	119	50.64	213	45.71	1		1	
CT	94	40	209	44.85	0.81 (0.58-1.12)	0.199	0.80 (0.57-1.12)	0.19
TT	22	9.36	44	9.44	0.90 (0.51-1.57)	0.697	0.87 (0.50-1.54)	0.634
CT+TT	116	49.36	253	54.29	0.82 (0.60-1.12)	0.217	0.81 (0.59-1.12)	0.199
CC+CT	213	90.64	422	90.56	1		1	
TT	22	9.36	44	9.44	0.99 (0.58-1.70)	0.973	0.97 (0.56-1.67)	0.906

[a]Adjusted for age, sex, smoking and drinking status; Bold values are statistically significant ($P < 0.05$)

and drinking status. The Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies to the expected ones among the control subjects. All statistical analyses were performed with SAS 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Characteristics of the study population

Characteristics of cases and controls included in the study are summarized in Table 1. The cases and controls appeared to be adequately matched on age

Table 4. Stratified Analyses Between BCL2: rs17757541 C>G Polymorphism and GCA Risk by Sex, Age, Smoking Status and Alcohol Consumption

Variable	BCL2: rs17757541 C>G (case/control)[a]				Adjusted OR[b] (95% CI); *P*				
	CC	CG	GG	CG+GG	CC	CG	GG	CG+GG	GG vs. (CC+CG)
Sex									
Male	93/206	57/73	2/5	59/78	1	**1.80 (1.17-2.77); *P*: 0.008**	0.82 (0.15-4.35); *P*: **0.811**	**1.73 (1.13-2.64); *P*: 0.011**	0.68 (0.13-3.60); *P*: 0.651
Female	60/132	19/45	4/4	23/49	1	0.79 (0.40-1.54); *P*: 0.483	2.48 (0.59-10.39); *P*: 0.215	0.92 (0.50-1.72); *P*: 0.799	2.61 (0.63-10.88); *P*: 0.187
Age									
<65	83/167	40/65	2/5	42/70	1	1.20 (0.74-1.96); *P*: 0.461	0.61 (0.12-3.26); *P*: 0.567	1.15 (0.71-1.86); *P*: 0.562	0.58 (0.11-3.06); *P*: 0.521
≥65	70/171	36/53	4/4	40/57	1	1.61 (0.97-2.69); *P*: 0.068	2.72 (0.65-11.35); *P*: 0.170	**1.69 (1.03-2.77); *P*: 0.040**	2.39 (0.58-9.88); *P*: 0.231
Smoking status									
Never	98/232	36/88	5/5	41/93	1	0.97 (0.61-1.54); *P*: 0.880	2.50 (0.70-8.88); *P*: 0.157	1.05 (0.67-1.64); *P*: 0.830	2.52 (0.71-8.91); *P*: 0.151
Ever	55/106	40/30	1/4	41/34	1	**2.45 (1.33-4.49); *P*: 0.004**	0.42 (0.04-4.20); *P*: 0.463	**2.19 (1.22-3.95); *P*: 0.009**	0.33 (0.03-3.20); *P*: 0.335
Alcohol consumption									
Never	110/241	47/92	5/6	52/98	1	1.04 (0.67-1.60); *P*: 0.867	1.86 (0.54-6.41); *P*: 0.325	1.09 (0.72-1.66); *P*: 0.693	1.84 (0.54-6.32); *P*: 0.330
Ever	43/97	29/26	1/3	30/29	1	**2.69 (1.39-5.19); *P*: 0.003**	0.84 (0.08-8.40); *P*: 0.881	**2.49 (1.31-4.74); *P*: 0.005**	0.62 (0.06-6.18); *P*: 0.687

[a]The genotyping was successful in 235 (96.7%) GCA cases, and 465 (97.7%) controls for BCL2: rs17757541 C>G; [b]Adjusted for age, sex, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model; Bold values are statistically significant (P <0.05)

and sex as suggested by the χ^2 tests (P = 0.923 and P = 0.197, respectively). As shown in Table 1, no significant difference was detected on drinking status between the cases and the controls (P = 0.217), but smoking rate was higher in GCA patients than in control subjects (P = 0.004). The primary information for six genotyped SNPs was in Table 2. For the six SNPs, the genotyping was successful ranging from 96.94% to 97.50% in all 719 samples. The concordance rates of repeated analyses were 100%. Minor allele frequency (MAF) in our controls was similar to MAF for Chinese in database for all six SNPs (Table 2). The observed genotype frequencies for these six polymorphisms in the controls were all in HWE (Table 2).

Associations between six polymorphisms and risk of GCA

The genotype distributions of *BCL2* rs17757541 C>G, *BCL2* rs12454712 T>C, *FAS* rs2234767 G>A, *FASL*/*FASLG* rs763110 C>T, *ERBB2* rs1136201 A>G and VEGFR2/KDR rs11941492 C>T in the cases and the controls are shown in Table 3. In the single locus analyses, the genotype frequencies of *BCL2* rs17757541 C>G were 65.11% (CC), 32.34% (CG), and 2.55% (GG) in the case patients and 72.69% (CC), 25.38% (CG), and 1.94% (GG) in the control subjects, and the difference was not statistically significant (P = 0.117). When the *BCL2* rs17757541 CC homozygote genotype was used as the reference group, the CG genotype was associated with a significantly increased risk for GCA (CG vs. CC: OR 1.42, 95% CI 1.01–2.01, P = 0.046). When the *BCL2* rs17757541 CC homozygote genotype was used as the reference group, the GG genotype was not associated with the risk for GCA (GG vs. CC: OR 1.47, 95% CI 0.52–4.21, P = 0.470). In the recessive model, when the *BCL2* rs17757541 CC/CG genotypes were used as the reference group, the GG homozygote genotype was not associated with the risk for GCA (OR 1.33, 95% CI 0.47–3.78, P = 0.595). In the dominant model, the *BCL2* rs17757541 CG/GG variants were associated with a significantly increased risk of GCA, compared with the *BCL2* rs17757541 CC genotype (CG/GG vs. CC: OR 1.43, 95% CI 1.02-2.00, P = 0.039) (Table 3). After adjusting for age, sex, smoking and drinking, a borderline statistically increased risk of GCA was observed in the heterozygote comparing model (CG vs. CC: adjusted OR 1.39, 95% CI 0.98–1.98, P = 0.063) and dominant model (CG/GG vs. CC: OR 1.40, 95% CI 0.99-1.96, P = 0.054) (Table 3).

None of the *BCL2* rs12454712 T>C, *FAS* rs2234767 G>A, *FASL*/*FASLG* rs763110 C>T, *ERBB2* rs1136201 A>G and VEGFR2/KDR rs11941492 C>T polymorphisms achieved a significant difference in the genotype distributions between cases and controls (Table 3). Logistic regression analyses revealed that the five polymorphisms were not associated with the risk of GCA (Table 3).

Stratification analyses of BCL2 rs17757541 C>G polymorphisms and risk of GCA

To evaluate the effects of *BCL2* rs17757541 C>G genotypes on GCA risk according to different age, sex, smoking and alcohol drinking status; we performed the stratification analyses. A significantly increased risk of GCA associated with the *BCL2* rs17757541 C>G polymorphism was evident among male patients, older patients and patients who smoking or drinking (Table 4).

Discussion

In this hospital-based case-control study of GCA, we investigated the associations of *BCL2* rs17757541 C>G, *BCL2* rs12454712 T>C, *FAS* rs2234767 G>A, *FASL*/*FASLG* rs763110 C>T, *ERBB2* rs1136201 A>G and

VEGFR2/KDR rs11941492 C>T SNPs with risk of GCA in a high risk Chinese population. Our analysis revealed that *BCL2* rs17757541 CG and CG/GG genotypes had an increased risk of GCA. The risk is evident among male patients, older patients and patients who smoking or drinking.

BCL2 is a proto-oncogene protein that inhibits apoptosis. The *BCL2* gene is located on chromosome 18q21.3 and consists of 3 exons and 2 promoters (Seto et al., 1988). SNPs in the *BCL2* gene, which alter protein function and/or expression, could impact the delicate balance of mechanisms regulating apoptosis. To date, lines of research have investigated the contributions of *BCL2* SNPs to the predisposition to different cancer types, such as papillary thyroid cancer (Eun et al., 2011), chronic lymphocytic leukemia (Enjuanes et al., 2008), squamous cell carcinoma of the head and neck (Chen et al., 2007), esophageal cancer (Jain et al., 2007), and so on. *BCL2* rs17757541 C>G polymorphism plays an important role in the development of younger-aged esophageal adenocarcinoma (Wu et al., 2011). However, no positive association was found between *BCL2* rs17757541 C>G SNP and GCA till now. We found *BCL2* rs17757541 CG variant heterozygote rather than *BCL2* rs17757541 GG homozygote was associated with GCA risk. This might because our sample size was relatively small and the numbers of GG genotypes were not large enough.

The frequencies of genetic polymorphisms often vary between ethnic groups. In the present Chinese study, the allele frequency of *BCL2* rs17757541 G was 0.146 among 476 control subjects, which is consistent with that of Chinese Han population (0.134) and European (0.158) population in SNP DataBase, but significantly higher than that of Sub-Saharan African (0.000) population and African ancestry in Southwest United States of America (0.020) (http://www.ncbi.nlm.nih.gov/SNP).

Considering *BCL2* rs17757541 C>G mutant alleles in the control group, OR, GCA samples and control samples, the power of our analysis ($\alpha = 0.05$) was 0.390 in 235 GCA cases and 465 controls with adjusted OR 1.42 for *BCL2* rs17757541 C>G.

Several limitations need to be addressed. The patients and controls were enrolled from hospitals and may not represent the general population. The polymorphisms investigated in our study were chosen based on their functional considerations, and may not give a comprehensive view about genetic variability in *BCL2*. Further fine-mapping studies in the susceptible region of the variants are needed. The small sample size limited the statistical power of our study. Further gene-environment interaction studies are warranted to clarify GCA carcinogenesis genetic mechanism. Detailed information on cancer metastasis and survival information were not available till now, which restricted us from further analyses on the role of *BCL2* polymorphisms in GCA progression and prognosis.

In conclusion, our study provides evidence that functional *BCL2* rs17757541 C>G polymorphism may contribute to the risk of GCA. However, our results were obtained with a limited sample size and were just preliminary conclusions; the power of our analysis was low. Future larger studies with more rigorous study designs of other ethnic populations and functional characterization are required to confirm current findings.

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