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Prostate stem cell antigen rs2294008 polymorphism differentially contributes to *Helicobacter pylori*-negative gastric cancer among various populations in China

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Abstract. Gastric cancer is a lethal disease with a high mortality rate. Studies have suggested that prostate stem cell antigen (PSCA) rs2294008 polymorphism is associated with gastric cancer (GC). In this case-control study, we investigated rs2294008 polymorphism in the Tibet, Hui and Han nationalities in the Qinghai area of China. Genomic DNA was extracted from the peripheral blood of 286, 315 and 350 healthy volunteers and from 219, 233 and 265 *Helicobacter pylori*-negative non-cardia GC patients from the Tibet, Hui and Han populations, respectively. The rs2294008 polymorphism was analyzed by denaturing high-performance liquid chromatography. rs2294008 CT and TT genotypes were associated with GC both in the Tibet and Han populations (adjusted OR=1.51, 1.47, 2.01, 1.85; 95% CI, 1.04-2.19, 1.05-2.06, 1.04-3.88, 1.03-3.34; P=0.030, 0.025, 0.039, 0.040, respectively). rs2294008 TT genotype was associated with GC in the Hui population (adjusted OR=2.14; 95% CI, 1.29-3.55; P=0.003). Furthermore, when stratified by histopathology, the rs2294008 CT and TT genotypes were associated with diffuse GC in the Tibet and Han nationalities (adjusted OR=1.93, 1.73, 2.69, 2.86; 95% CI, 1.09-3.44, 1.01-2.95, 1.06-6.84, 1.27-6.46; P=0.025, 0.045, 0.038, 0.011, respectively). However, the rs2294008 TT genotype was associated with both intestinal and diffuse types of GC (adjusted OR=2.10, 2.21; 95% CI, 1.17-3.75, 1.12-4.38; P=0.012, 0.023, respectively) and the rs2294008 CT genotype was only associated with intestinal-type GC in the Hui nationalitiy group (adjusted OR=1.60; 95% CI, 1.04-2.47; P=0.034). The results therefore showed that rs2294008 may differentially contribute to GC among different nationalities in one area and its role is independent from *Helicobacter pylori*-infection.

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Introduction

Gastric cancer (GC) is estimated to be the fourth most common cancer and the second leading cause of cancer-related death worldwide, with ~40% GC cases occurring in China (1). The mortality of GC in China is the highest in the world, especially in the northwestern part of the country, which includes the Qinghai province (2,3). Han nationality constitutes the majority population, while the Tibet and Hui nationalities are considered minorities. The incidence of GC in the Tibet and Hui populations is higher than that in the Han nationality. However, the study of the Tibet and Hui nationalities is not as advanced due to fewer individuals and poorer economic conditions (4).

Previous studies demonstrated that environmental factors (5), bacterial infection (*Helicobacter pylori*, *H. pylori*) (6) and genetic factors (7) are important in GC development. Although the strongest known risk factor for non-cardia GC is *H. pylori* infection and there is a high rate of *H. pylori* infection in Eastern Asians (8,9), only a small proportion of *H. pylori*-infected individuals is likely to develop neoplasia. This is probably due to gastric carcinogenesis depending on specific combinations of bacterial strains, environmental factors and host genetic susceptibility factors (10,11).

Findings of recent studies have detected the associations between genetic polymorphisms and risk of GC (12,13). In 2008, the Study Group of the Millennium Genome Project for Cancer found an association between the rs2294008 single nucleotide polymorphism (SNP) in the prostate stem cell antigen gene (PSCA) and the risk of GC in Japanese and Korean populations (14). The association between GC and rs2294008 has also been identified in Chinese Han and Caucasian populations (15,16).

Absence of concomitant *H. pylori* infection may be a factor confounding the contribution of gene variants to disease (17,18). Moreover, certain gene polymorphisms have been confirmed to differentially affect GC development among various populations (19). Therefore, a case-control study was conducted to investigate the potential etiologic role of rs2294008 polymorphism in *H. pylori*-negative patients from the Tibet, Hui and Han nationalities in the Qinghai region of China.

Materials and methods

Study subjects. Consecutive healthy controls and patients with *H. pylori*-negative, non-cardia cancer were enrolled. The Tibet, Hui and Han nationalities from the Qinghai Province of China were selected for this study. This case-control study was approved by the institutional review board of Qinghai University. Healthy controls included 286 Tibet, 315 Hui and 350 Han individuals enrolled from the Hainan Tibet Nationality Autonomous Prefecture, Minhe Hui Nationality Autonomous County and Xining city in the Qinghai province, respectively. Between January, 2009 and May, 2012, 219, 233 and 265 Tibet, Hui and Han individuals, respectively, suffering from GC were enrolled in this study from the Affiliated Hospital of Qinghai University. Recruited healthy controls were from families that had a history of longevity in that locality, did not marry other nationalities for at least three generations and did not have blood relations with each other. Age, gender and smoking status of healthy controls were matched to patients and are shown in Table I. The GC cases were histopathologically diagnosed and the exclusion criteria of GC cases included having a history of any cancer or any metastasized cancer (carcinomas were not originally from stomach) and having undergone radiotherapy or chemotherapy. Subjects with a family history of any cancer were also excluded. The patients were histologically confirmed as having non-cardia GC. Subjects who consented to participate in the study and donate blood samples were included in this study. Each subject was personally (face-to-face) interviewed by trained interviewers, using a pretested questionnaire to obtain information on demographic data and smoking habits. The presence of *H. pylori* infection in the sera of patients and controls was measured with an enzyme-linked immunosorbent assay (anti-*H. pylori* enzyme immunoassay; Huamei Biotech Inc., Wuhan, China).

Genotype analyses. Genomic DNA was isolated from 5 ml of venous blood by the conventional proteinase K digestion and phenol/chloroform extraction method. Polymorphism was analyzed by PCR-based denaturing high-performance liquid chromatography (DHPLC). Primers were synthesized at the Beijing Aoke Biological Engineering Technology and Services (Beijing, China), and are shown together with PCR conditions, PCR annealing temperatures and DHPLC detection methods in Table II. PCR was performed with a 25 ml reaction mixture containing 100 ng of genomic DNA, 1.0 mM of primer, 0.2 mM of dNTP, 2.0 mM of $MgCl_2$ and 1.0 Taq units DNA polymerase in 1X reaction buffer (Promega, Madison, WI, USA). DHPLC analysis was performed on a Transgenomic WAVE® System. The detailed genotyping process has been previously described (20). The PCR products were applied to the DHPLC column at an optimal oven temperature and eluted with a linear acetonitrile gradient at a flow rate of 0.9 ml/min. The genotypes identified by DHPLC analysis were confirmed by DNA sequencing with the ABI Prism 377 DNA Sequencer.

Helicobacter pylori antibody assays. Enzyme-linked immunosorbent assay for the detection of *H. pylori* was performed as per the manufacturer's instructions. Following termination of the enzyme reaction, absorbance at 630 nm was measured. Absorbance ratios (sample/negative control) ≥2.1 were considered positive while ratios of <2.1 were considered negative.

Table I. Select characteristics and risk factors in patients with gastric cancer and controls from the Tibet, Hui and Han nationalities.

	Tibet				Hui				Han			
Variables	Cases (%) (n=219)	Controls (%) (n=286)	χ^2	P-value	Cases (%) (n=233)	Controls (%) (n=315)	χ^2	P-value	Cases (%) (n=265)	Controls (%) (n=350)	χ^2	P-value
Age (years)												
<35	4 (1.82)	6 (2.10)	0.047	0.828	3 (1.29)	3 (0.95)	0.139	0.709	7 (2.64)	8 (2.29)	0.080	0.777
35-60	116 (52.97)	152 (53.15)	0.002	0.968	112 (48.07)	157 (49.84)	0.168	0.682	132 (49.81)	182 (52.00)	0.289	0.591
≥60	99 (4521)	128 (44.75)	0.010	0.920	118 (50.64)	155 (49.21)	0.111	0.739	126 (47.55)	160 (45.71)	0.203	0.652
Gender												
Male	162 (73.97)	209 (73.08)	0.051	0.821	173 (74.25)	223 (70.79)	0.798	0.372	198 (74.72)	262 (74.86)	0.001	0.968
Female	57 (26.27)	77 (26.92)			60 (25.75)	92 (29.21)			67 (25.28)	88 (25.14)		
Smoking												
Yes	140 (63.93)	186 (65.03)	0.067	0.796	42 (18.03)	62 (19.68)	0.239	0.625	175 (66.04)	221 (63.14)	0.551	0.511
No	79 (36.07)	100 (34.97)			191 (81.97)	253 (80.32)			90 (33.96)	129 (36.86)		

Table II. Primer sequences, PCR and DHPLC conditions for the detection of gene polymorphisms.

Gene	Primer sequence	PCR annealing temperature (˚C)	PCR product size (bp)	DHPLC application type	Oven temperature (˚C)
PSCA	F: AGTCACCTGAGGCCCTCTC R: CTGCAGCCTTTGCTGATGACG	59	241	Mutation	59

PCR, polymerase chain reaction; DHPLC, denaturing high-performance liquid chromatography.

Statistical analysis. Data were analyzed using SPSS software version 17.0 (SPSS, Chicago, IL, USA). The significance of the difference in the distribution of the polymorphisms among different groups was calculated using the χ^2 test. The allelic distributions were examined for deviations from their corresponding Hardy-Weinberg equilibrium. Multivariate logistic regression was used to obtain odds ratios (ORs) and 95% confidence intervals (CIs), adjusting for age, gender and smoking status. P<0.05 (two-tailed) was considered to indicate a statistically significant difference.

Results

Clinical characteristics. The study population comprised 286 healthy controls and 219 GC patients from the Tibet nationality, 315 healthy controls and 233 GC patients from the Hui nationality and 350 healthy controls and 265 GC patients from the Han nationality. Age, gender and smoking status of the GC patients and control subjects are shown in Table I. The healthy controls and GC patients were consecutive *H. pylori*-negative. No statistically significant differences were identified between the cases and controls among age, gender and smoking status in each nationality group. The genotype frequencies of rs2294008 in the controls in each nationality group were in agreement with the Hardy-Weinberg equilibrium (P>0.05 for all).

PSCA rs2294008 gene polymorphism and association with GC. The frequencies of genotypes rs2294008 gene polymorphism among the Tibet, Hui and Han nationalities are summarized in Table III. In the Tibet nationality group, the rs2294008 CT genotype was significantly more frequent in GC patients (47.03%) compared with controls (39.51%) (χ^2=4.75, P=0.030). The risk of developing GC with this genotype was significantly increased (adjusted OR=1.51; 95% CI, 1.04-2.19; P=0.030). The rs2294008 TT genotype also occurred more frequently in GC patients (10.50%) compared with controls (6.64%) (χ^2=4.37, P=0.039). The risk of developing GC with this genotype was also significantly increased (adjusted OR=2.01; 95% CI, 1.04-3.88; P=0.030).

Unlike the results obtained from individuals belonging to the Tibet populations, in the Hui nationalitiy group, there were no differences in the frequencies of the rs2294008 CT genotypes between GC patients and controls (χ^2=2.82, P=0.093). However, the rs2294008 TT genotype was identified more frequently in GC patients (21.03%) compared with controls (13.02%) (χ^2=8.88, P=0.003). The risk of developing GC with this genotype was significantly increased (adjusted OR=2.14; 95% CI, 1.29-3.55; P=0.003).

Results obtained from the Tibet region showed two genotype sites were associated with GC in the Han nationality. For the rs2294008 CT genotype, there was a significant difference between GC patients (47.92%) and controls (40.86%) (χ^2=4.61, P=0.025). The risk of developing GC with rs2294008 CT genotypes was significantly increased (adjusted OR=1.47; 95% CI, 1.05-2.06; P=0.025). In addition, for the rs2294008 TT genotype, there was a statistically significant difference in the TT genotype distribution between GC (10.57%) and controls (7.14%) (χ^2=8.70, P=0.040). The risk of developing GC with rs2294008 TT genotype was significantly increased (adjusted OR=1.85, 3.53; 95% CI, 1.03-3.34; P=0.004).

Association of PSCA rs2294008 with clinicopathological features. Stratified analyses were performed according to histopathology (Lauren's classification) with adjustment for age, gender and smoking status. The prevalence of rs2294008 polymorphism in different GC subtypes was analyzed. In this study, gastric carcinomas were of intestinal histotype in 153 (69.86%), 156 (66.95%) and 188 patients (70.94%) and of diffuse histotype or mixed-type GC in 66 patients (30.14%), 77 (33.05%) and 77 patients (29.06%) from the Tibet, Hui and Han nationalities, respectively. The frequencies of rs2294008 genotypes among intestinal-type and diffuse or mixed-type GC from the three populations are recorded in Table IV.

Similar results were observed in both the Tibet and Han nationality groups. For individuals belonging to the Tibet nationality group, the rs2294008 CT and TT genotypes was only associated with diffuse GC (P=0.025 and 0.038, respectively) with adjusted OR of 1.93 (95% CI, 1.09-3.44) and 2.69 (95% CI, 1.06-6.84). However, the rs2294008 CT and TT genotypes were not associated with intestinal-type GC (P=0.142 and 0.129, respectively; adjusted OR=1.36; 95% CI, 0.90-2.06; 1.76, 0.85-3.69, respectively) (Table IV). Additionally, for individuals belonging to the Han nationality group, the rs2294008 CT and TT genotypes were also only associated with diffuse GC (P=0.045 and 0.011, respectively) with adjusted OR of 1.73 (95% CI, 1.01-2.95) and 2.86 (95% CI 1.27-6.46). However, the rs2294008 CT and TT genotypes were not associated with intestina-type GC (P=0.088 and 0.228, respectively; adjusted OR=1.38; 95% CI, 0.95-2.00; 1.51, 0.77-2.95, respectively).

Unlike the results obtained from individuals belonging to the Tibet and Han populations, the rs2294008 TT genotype in the Hui nationalitiy group, was associated with both intestinal and diffuse types of GC (P=0.012 and 0.023, respectively) with adjusted ORs of 2.10 and 2.21 (95% CI, 1.17-3.75 and 1.12-4.38, respectively). Nevertheless, the rs2294008 CT genotype was only associated with intestinal-type GC (P=0.034) with an

Tables III. Genotype distributions of rs2294008 polymorphism among gastric cancer cases and controls from the Tibet, Hui and Han nationalities.

Genotype	Cases, n (%)	Controls, n (%)	OR (95% CI)[a]	P-value
Tibet	219	286		
CC	93 (42.47)	154 (53.85)	1	
CT	103 (47.03)	113 (39.51)	1.51 (1.04-2.19)	0.030
TT	23 (10.50)	19 (6.64)	2.01 (1.04-3.88)	0.039
Hui	233	315		
CC	72 (30.90)	129 (40.95)	1	
CT	112 (48.07)	145 (46.03)	1.38 (0.95-2.02)	0.093
TT	49 (21.03)	41 (13.02)	2.14 (1.29-3.55)	0.003
Han	265	350		
CC	110 (41.51)	182 (52.00)	1	
CT	127 (47.92)	143 (40.86)	1.47 (1.05-2.06)	0.025
TT	28 (10.57)	25 (7.14)	1.85 (1.03-3.34)	0.040

[a]Adjusted for age, gender and smoking status.

Table IV. Genotype distributions of the rs2294008 polymorphism among different subtypes of gastric cancer from the Tibet, Hui and Han nationalities.

		Intestinal cases			Diffuse cases		
Genotype	Controls, n (%)	Cases	OR (95% CI)[a]	P-value	Cases	OR (95% CI)[a]	P-value
Tibet							
CC	154 (53.85)	69 (45.10)	1		24 (36.36)	1	
CT	113 (39.51)	69 (45.10)	1.36 (0.90-2.06)	0.142	34 (51.52)	1.93 (1.09-3.44)	0.025
TT	19 (6.64)	15 (9.80)	1.76 (0.85-3.69)	0.129	8 (12.12)	2.69 (1.06-6.84)	0.038
Hui							
CC	129 (40.95)	45 (28.85)	1		27 (35.06)	1	
CT	145 (46.03)	81 (51.92)	1.60 (1.04-2.47)	0.034	31 (40.26)	1.02 (0.58-1.18)	0.941
TT	41 (13.02)	30 (19.23)	2.10 (1.17-375)	0.012	19 (24.68)	2.21 (1.12-4.38)	0.023
Han							
CC	182 (52.00)	82 (43.62)	1	0.005	28 (36.36)	1.79 (0.85-3.77)	0.126
CT	143 (40.86)	89 (47.34)	1.38 (0.95-2.00)	0.088	38 (49.35)	1.73 (1.01-2.95)	0.045
TT	25 (7.14)	17 (9.04)	1.51 (0.77-2.95)	0.228	11 (14.29)	2.86 (1.27-6.46)	0.011

[a]Adjusted for age, gender and smoking status.

adjusted OR of 1.60 (95% CI, 1.04-2.47) and was not associated with diffuse GC (P=0.941; adjusted OR=1.02; 95% CI, 0.58-1.8). No other significant associations were found when GC patients were classified according to age, gender, or smoking status.

Discussion

To the best of our knowledge, this is the first study examining the associations between rs2294008 polymorphism and GC risk among several nationalities in one region at the same time and also the first study to investigate the association between rs2294008 polymorphism and GC in a Hui population. In this case-control study, we investigated the association between rs2294008 polymorphism and *H. pylori*-negative non-cardia GC among three nationalities, including Tibet, Hui and Han nationalities, in the Qinghai area of China. We found that rs2294008 CT and TT were associated with a significantly increased risk of GC in both the Tibet and Han nationalities, while the rs2294008 TT genotype was associated with a significantly increased risk of GC in the Hui nationalitiy. Additionally, we found that rs2294008 CT and TT were associated with a significantly increased risk of diffuse GC only in the Tibet and Han populations, whereas the rs2294008 TT genotype was associated with intestinal and diffuse GC in the Hui population, with the rs2294008 CT genotype being associated only with intestinal-type gastric in this population. These findings indicate that rs2294008 may contribute to the etiology of gastric carcinogenesis and rs2294008 may differ-

entially contribute to GC among various nationalities in one region and its roles are independent from *H. pylori* infection.

PSCA maps on chromosome 8q24.2 (21), comprising 3 exons and 2 introns. PSCA is a member of the LY-6/Thy-1 family of glycosylphosphatidylinosi-tol-anchored cell surface proteins and is a human cancer marker closely related to stem cell antigens (21,22). PSCA was overexpressed in prostate (23) and pancreatic cancer, (24), however, its expression decreased in head and neck squamous cell carcinoma (25), as well as urothelium, kidney, esophageal, bladder and stomach cancer (26,27,14). Previous studies have shown that PSCA may be involved in signal transduction and cell growth regulation in various systems (28,29). In 2008, a two-stage genome-wide association study of GC in Japanese and Korean populations, identified a SNP 2294008, in exon 1 of PSCA, associated with susceptibility to diffuse GC (14). The authors noted that the risk allele 'T' of rs2294008 reduced transcriptional activity of an upstream fragment of the gene. Recently, this allele has been identified to be associated with susceptibility to GC risk in Chinese Han and Caucasian populations (15,16). At least two meta-analyses on associations between PSCA polymorphisms and GC have been reported. Findings of meta-analysis examining nine case-control studies comprising 10,746 cases and 9,158 controls demonstrated that the PSCA rs2294008 C-T polymorphism exhibited a significantly increased risk of GC in the genetic models (TT/TC vs. CC: OR=1.61; 95% CI, 1.35-1.91; TT vs. TC/CC: OR=1.33; 95% CI, 1.24-1.42) and that this polymorphism may contribute to susceptibility to GC, particularly in non-cardia or diffuse GC. Furthermore, in the stratified analysis ethnicity of rs2294008, an increased GC risk was found in both Asians (TT vs. TC/CC: OR=1.31; 95% CI, 1.22-1.42) and Europeans (TT/TC vs. CC: OR=1.42; 95% CI, 1.18-1.71) (30). Another meta-analysis that included eight case-control studies in seven articles comprising 9,738 cases of GC and 7,054 controls indicated that the T allele of rs2294008C>T was significantly associated with increased GC risk [rs2294008C>T: OR (95% CI)=1.31 (1.22-1.42), Pz-test <0.001]. Results of the subgroup analyses indicated that T allele of rs2294008C>T was associated with increased risk of both intestinal and diffuse GC, GC for Eastern Asian (including Chinese, Japanese, Koreans, PCC and HCC/PHCC), cardia and non-cardia GC and GC for males and females (31).

Our study suggests that carriers of the PSCA rs2294008 CT and TT genotypes had increased risks of GC in the Tibet and Han nationalities in the Qinghai area of China. These results are consistent with most studies on Chinese Han and Tibet, as well as Japanese, Korean and Caucasian populations, were rs2294008 CT and TT genotypes were found to be associated with an increased risk of GC (16,32-35). However, only rs2294008 TT genotypes had an increased risk of GC in the Hui nationality in contrast to the Tibet and Han nationalities. Associations between rs2294008 and GC risk among the Tibet, Han and Hui nationalities were consistent regardless of age, gender or smoking status. The patients and controls enrolled in this study were *H. pylori*-negative and matched with regards to age, gender and smoking status. Therefore, our results suggest that rs2294008 was an independent risk factor of GC, which is in concordance with previous studies (16,34).

Of note, when GC cases were subclassified according to their histological type (intestinal, diffuse or mixed) the association between rs2294008 and GC risk among the Tibet, Hui and Han nationalities was not identical. rs2294008 CT and TT genotypes were associated with diffuse but not intestinal-type GC in the Tibet and Han nationality groups. These findings are in agreement with those previously reported, including studies from Japanese and Caucasian populations (14,16). Nevertheless, the rs2294008 TT genotype was associated with both intestinal and diffuse types of GC, while the rs2294008 CT genotype was only associated with intestinal-type GC in the Hui nationalitiy group. A similar finding was observed in two Chinese Han populations (32,36).

Descrepancies in the association between rs2294008 genotypes and GC among the Tibet, Hui and Han nationalities are associated with different inherited gene backgrounds. Geographically restricted positive selection due to distinct environmental pressures often results in large allele frequency differences between populations. Studies have shown that differential genetic/environmental interactions in different ethnic groups resulted in altered gene expression and altered effects on cell growth and the development of GC (19,37). The gene distribution of rs2294008 among the Tibet, Hui and Han nationalities in Qinghai was not identical. Our data might reflect the effect of past selective pressures on genotypes of Tibet, Hui and Han nationalities over a long period of time. The rs2294008 CT and TT genotypes were similar in the Tibet (39.51%, 6.64, respectively) and Han (40.86%, 7.14, respectively) populations, but were significantly lower than those of the Hui population (46.03%, 13.02, respectively). The Han nationality constitutes the majority population, while Tibetans are a minority in the Qinghai area in China, with both groups having resided in a high-altitude area for a long period of time, thus their physical and physiological functions have altered to adapt to hypoxic environments (38). A recent study has identified that advantageous gene variations are associated with human adaptation at high altitudes (39), thus, genetic susceptibility of the Tibet and Han populations may yield different results from Han populations only. The Hui nationality, which migrated from Central Asia, Persia and the Arab world, is also a minority in the Qinghai area in China. A study analyzed M*, N* and R* mtDNAs and found that the western Eurasian specific haplogroup frequency in the Hui population was 6.7% but no western Eurasian type was found in Han Chinese samples from the same region (40). The Hui nationality has its own gene characteristics. Both the Tibet and Hui nationalities carry out endogamy and tend to be ethnically homogeneous. Therefore, the Tibet, Hui and Han nationalities in the Qinghai area in China likely have different gene traits leading to different associations between PSCA rs2294008 polymorphism and GC risk.

The present study was limited in patient and control size. Additionally, factors including education or consumption of alcohol, fresh fruits or vegetables in the controls were not considered, while varying eating habits among patients and controls were not investigated. In general, the Tibet nationality diet contains more meat and lacks certain nutrients, may potentially increasing GC risk. The Hui nationality observes Ramadan and eats refrigerated foods, potentially causing gastric mucosa pathological changes.

In conclusion, the present study has shown different associations of the rs2294008 polymorphism and GC risk among the Tibet, Hui and Han nationalities in the Qinghai area of

China. rs2294008 CT and TT genotypes were associated with diffuse GC in the Tibet and Han nationalities. Although the rs2294008 TT genotype was associated with both intestinal and diffuse types of GC, the rs2294008 CT genotype was only associated with intestinal-type GC in the Hui nationalitiy group. These results demonstrate that rs2294008 may differentially contribute to GC among different nationalities in a particular region and its roles are independent of *H. pylori* infection.

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