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Genetic Variants in T Helper Cell Type 1, 2 and 3 Pathways and Gastric Cancer Risk in a Polish Population

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Host immune responses are known determinants of gastric cancer susceptibility. We previously reported an increased gastric cancer risk associated with common variants of several T helper type 1 (Th1) cytokine genes in a population-based case–control study in Warsaw, Poland. In the present study, we augmented our investigation to include additional Th1 genes as well as key genes in the Th2 and Th3 pathways. Analysis of 378 cases and 435 age- and sex-matched controls revealed associations for polymorphisms in the Th1 *IL7R* gene and one polymorphism in the Th2 *IL5* gene. The odd ratios (ORs) for *IL7R* rs1494555 were 1.4 [95% confidence interval (CI), 1.0–1.9] for A/G and 1.5 (95% CI, 1.0–2.4) for G/G carriers relative to A/A carriers ($P = 0.04$). The ORs for *IL5* rs2069812 were 0.9 (95% CI, 0.7–1.3) for C/T and 0.6 (95% CI, 0.3–1.0) T/T carriers compared with C/C carriers ($P = 0.03$). These results suggest that *IL5* rs2069812 and *IL7R* rs1389832, rs1494556 and rs1494555 polymorphisms may contribute to gastric cancer etiology.

Key words: gastric cancer – T helper cell pathways – polymorphism

INTRODUCTION

Helicobacter pylori infection is a major risk factor for gastric cancer (1–3). The infection usually causes chronic gastritis, but only a small proportion of the infected subjects develop gastric cancer (1,4). Host immune responses are known determinants of gastric cancer susceptibility (3,5,6). Cytokines produced by a variety of activated T cells act as regulators and mediators of immune responses. Cytokines have been classified into several different groups based on their functions, including T helper type 1 (Th1) pro-inflammatory cytokines, such as IL2, IL12, IFN and TNFα; T helper type 2 (Th2) anti-inflammatory cytokines, such as IL4, IL6, IL10 and T helper type 3 (Th3) regulatory cytokine such as TGF (7).

Most data have suggested that *H. pylori* infection triggers a predominant Th1 response (3,8), which has been demonstrated to promote gastric atrophy and carcinogenic processes (9,10). The role of Th2 cytokines in *H. pylori* infection-related gastric carcinogenesis is less studied (10), and there are conflicting reports regarding their etiologic role. On the one hand, a shift toward a Th2 response against *H. pylori* has been shown to favor gastric mucosal protection, with a clearance of infection (11). On the other hand, a shift from a Th1 to a Th2 cell

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pattern had been linked to gastric carcinoma (12,13). It is now well-established that immune responses driven by Th1 and Th2 cells can be influenced by Th3 cells. Th3 cells produce high levels of TGF, whose main function is counter-regulation or suppression of immune responses mediated by Th1 and Th2 cells (14,15). Th3 cells that produce TGF have been observed in experimental models of colitis, diabetes and $HgCl_2$-induced autoimmune disease and are believed to play a role in the prevention and treatment of these diseases (16–18). Since genetic control plays a role in modulating cytokine production (19,20), we hypothesize that inter-individual variations in single nucleotide polymorphism (SNP) profiles may contribute to variability in immune responses, leading to variability in outcomes. We previously reported an increased risk of gastric cancer associated with polymorphisms of TNFA-308 A>G and IFNGR2 Ex7-128 C>T, IL-1B-31, IL-1B-511 and IL-1RN *2/*2 may increase the risk of gastric cancer in a high-risk Polish population (5,21).

In the present study, three additional Th1 genes (i.e. *IL7R*, a membrane receptor for the Th1 cytokine interleukin-7, *IL15 and LTA, two* pro-inflammatory cytokines) were examined. We also examined five *Th2* genes, including *IL4, IL5, IL6, IL10* and *IL13*. IL4 plays a key role in the differentiation of helper T cells to the Th2 type, regulates production of IgE by B lymphocytes and regulates mast cell expression of leukotriene C4 synthase (22,23). IL4 levels were correlated with *H. pylori* infection and levels of mucosal anti-*H. pylori* immunoglobulin G in gastric biopsy culture supernatants from gastric cancer patients (22,23). Increased IL4 has been detected in antral tissue of *H. pylori* infected adults (24). IL5 is a major determinant of the Th2 response and the serum levels of IL5 have been found to be increased in *H. pylori*-infected subjects with chronic gastritis, a precursor to gastric cancer (25). IL6 is a key mediator of the Th2 response and increased levels of IL6 are associated with worsening prognosis in advanced gastric cancer (26). IL10 regulates B-cell proliferation and differentiation (27). Lower IL10 levels could result in higher TNFα expression, shifting the balance toward Th2 humoral immunity (28). IL13 has been shown to inhibit the production of other pro-inflammatory cytokines (29).

In addition, we also examined one Th1/Th2 gene, *CTLA4*, an essential inhibitor of T-cell activity and thus is a strong candidate gene for studies of autoimmune and inflammatory diseases (30), and one Th3 gene, *TGF1*, a growth factor that normally inhibits cell growth, but in cancer cells enhances tumor progression and metastases (31).

The selection of the genes included in the present study was based on the biological function of these genes, and previous literatures report that polymorphisms in these genes are associated with cancer risks or chronic infectious conditions.

SUBJECTS AND METHODS

Subjects

The present population-based case–control study of gastric cancer was carried out in Warsaw, Poland, between 1994 and 1996. The study population has been described in detail previously (21,32,33). Residents aged 21–79 years who were newly diagnosed with gastric cancer (ICD-O 151 or ICD-O-2 C16) were identified by collaborating physicians in each of the 22 hospitals serving the Warsaw area. All diagnoses were pathologically confirmed by study pathologists. Controls were randomly selected among Warsaw residents from a computerized registry of all legal residents in Poland, and were frequency-matched to cases by sex and age in 5-year groups. The registry was updated monthly, and the completeness of registration was estimated to be nearly 100%.

The study protocol was approved by all appropriate institutional review boards. Detailed information on demographic characteristics and history of exposures to potential risk factors was recorded during a personal interview, after written consent was obtained. Among the 464 gastric cancer cases and 480 controls eligible for the study, genomic DNA from blood was obtained from 378 (81.5%) cases and 435 (90.1%) controls.

Genotyping

We assessed 12 SNPs in three Th1-related genes, 10 SNPs in five Th2-related genes, 1 SNP in a gene related to both Th1 and Th2 pathways and 3 SNPs in a gene related to the Th3 pathway. Non-synonymous and promoter SNPs were selected based on the literature evidence regarding the function and disease associations of each polymorphism. Synonymous SNPs in coding and non-coding regions were also selected to improve coverage of candidate genes. TaqMan™ (Applied Biosystems, Inc. Foster City, CA, USA) and MGB Eclipse™ assays were used to assess SNPs in *IL7R* [IVS1+1560 G>A, rs1389832; IVS3+1472 A>C, rs1494556; Ex4+33 A>G (I138V), rs1494555; Ex4-43 C>T (H165H), rs7737000], *LTA* [Ex1+49 C>A, rs2239704; IVS1+90 A>G, rs909253; IVS1-82 G>C, rs746868; Ex2+46 T>C (C13R), rs2229094], *IL15* (Ex3-92 C>T, rs2254514; Ex9-181 T>A, rs1057972; Ex9-95 G>T, rs9282743; Ex9-66 C>T, rs10833), *IL5* (-745 C>T, rs2069812), *IL10* (−3584 T>A, rs1800890), *IL13* (IVS3-24 C>T, rs1295686; −1069 C>T, rs1800925; Ex4+98 G>A, rs20541), *CTLA4* [Ex1-61 A>G (T17A), rs231775], and *TGF1* [−509 C>T, rs1800469; Ex1-327 T>C (L10P), rs1982073; Ex5-73 C>T (T263I), rs1800472]. These assays were conducted at the Core Genotyping Facility (CGF), National Cancer Institute and National Institutes of Health (NIH). Assays were validated and optimized as described in the SNP500 Cancer website (34). Assay-specific primer/probe concentrations and thermo-cycling conditions for these SNPs are available on the CGF website (http://snp500cancer.

nci.nih.gov). For each genotype, as a lab internal quality control, four human DNA controls (Coriell DNA) as well as no template controls were run with study samples. Approximately 8% blind quality control samples from two individuals were interspersed with the study samples, showing >99% concordance. Genotyping data for each tested SNP were successfully obtained for ≥95% of the subjects. The remaining five SNPs, *IL4* (−589 C>T, rs2243250), *IL6* (−174 G>C, rs1800795) and *IL10* (−1082 A>G, rs1800896 and −592 C>A, rs1800872), were also determined by 5′-nuclease polymerase chain reaction assays (TaqMan™) using methods as described previously (5,35) at the Viral Epidemiology Section, Science Applications International Corporation-Frederick, NIH. The sequences of the primers and probes used for these SNPs in the TaqMan™ assays are provided elsewhere (35).

Statistical Methods

Hardy–Weinberg equilibrium in controls was confirmed for all SNPs using the asymptotic Pearson's chi-square test. The Fisher's exact test was used to assess the difference in the distributions of categorical variables between cases and controls. Unconditional logistic regression was conducted using Stata version 8 (Stata Corporation, College Station, TX, USA) to calculate odd ratios (ORs) and 95% confidence intervals (CIs) for gastric cancer in association with specific genotypes. For all genotypes, the homozygote of the common allele was used as the reference group. Haplotype analysis was conducted on genes for which multiple SNPs were genotyped, including *IL15*, *LTA*, *IL7R*, *IL10*, *IL13*, *IL15*, *TGF1*, as well as for genes in close proximity to each other, such as *IL4*, *IL5* and *IL13*. HaploView was used to assess pairwise linkage disequilibrium between polymorphisms within each gene (36). Haplotypes were constructed from genotype data by means of the Haplo.Stats package (R version 2.2.1) (37). All ORs were adjusted for age, gender, education and smoking. Further adjustment for other potential confounding variables, including family history of cancer, pack-years of cigarette smoking, dietary intake and use of ulcer medications, did not affect the risks meaningfully. We tested the statistical significance of multiplicative gene–gene interaction terms using the likelihood ratio test, comparing logistic regression models with and without the appropriate interaction term. All tests were two-sided at the 0.05 significance level.

RESULTS

Demographic characteristics of the study population have been published previously (33).

Genotype distribution for each assessed SNP was in Hardy–Weinberg equilibrium among controls. The associations between SNPs tested and gastric cancer risk are shown in Table 1. Of the Th1 SNPs tested, three in *IL7R* were

Table 1. Gastric cancer risk and polymorphisms in genes in Th1, Th2 and Th3 pathways in a Polish population

	Cases ($n = 378$)[1]	Controls ($n = 435$)[1]	OR[2]	95% CI	*P* value
Th1 genes					
IL15 Ex3-92 C>T (rs2254514)					
CC	167	221	1.0		
CT	107	155	0.9	0.6–1.2	
TT	23	26	1.2	0.6–2.1	0.82
IL15 Ex9-181 T>A (rs1057972)					
TT	80	101	1.0		
TA	150	203	0.9	0.6–1.3	
AA	67	99	0.8	0.5–1.3	0.35
IL15 Ex9-95 G>T (rs9282743)					
GG	248	323	1.0		
GT	47	73	0.8	0.5–1.2	
TT	2	3	0.7	0.1–4.3	0.29
IL15 Ex9-66 C>T (rs10833)					
CC	139	203	1.0		
CT	133	159	1.2	0.9–1.7	
TT	21	40	0.8	0.4–1.4	0.95
LTA Ex1+49 C>A (rs2239704)					
CC	85	105	1.0		
CA	138	223	0.8	0.5–1.1	
AA	76	85	1.1	0.7–1.6	0.87
LTA IVS1 + 90 A>G (rs909253)					
AA	137	201	1.0		
AG	135	174	1.1	0.8–1.6	
GG	29	38	1.1	0.7–2.0	0.44
LTA IVS1-82 G>C (rs746868)					
GG	83	108	1.0		
GC	143	220	0.9	0.6–1.2	
CC	74	84	1.1	0.7–1.7	0.72
LTA Ex2 + 46 T>C (C13R) (rs2229094)					
TT	206	247	1.0		
TC	74	150	0.6	0.4–0.8	
CC	21	18	1.6	0.8–3.1	0.22
IL7R IVS1+1560 G>A (rs1389832)					
GG	105	175	1.0		
GA	141	177	1.3	0.9–1.9	
AA	55	60	1.6	1.0–2.6	0.03
IL7R IVS3+1472 A>C (rs1494556)					
AA	106	181	1.0		
AC	142	174	1.4	1.0–1.9	
CC	52	56	1.7	1.1–2.7	0.01
IL7R Ex4 + 33 A>G (I138V) (rs1494555)					
AA	107	177	1.0		

Continued

Table 1. *Continued*

	Cases ($n = 378$)[1]	Controls ($n = 435$)[1]	OR[2]	95% CI	*P* value
AG	141	169	1.4	1.0–1.9	
GG	51	58	1.5	1.0–2.4	0.04
IL7R Ex4-43 C>T (H165H) (rs7737000)					
CC	242	323	1.0		
CT	56	81	0.9	0.6–1.4	
TT	3	6	0.7	0.2–3.1	0.58
Th2 genes					
IL4-589 C>T (rs2243250)					
CC	241	278	1.0		
CT	99	133	0.8	0.6–1.2	
TT	16	14	1.4	0.6–2.9	0.79
IL5-745 C>T (rs2069812)					
CC	161	210	1.0		
CT	115	163	0.9	0.7–1.3	
TT	20	44	0.5	0.3–1.0	0.03
IL6-174 G>C (rs1800795)					
GG	98	104	1.0		
CG	164	197	0.9	0.6–1.2	
CC	80	87	1.0	0.6–1.5	0.88
IL10-592 C>A (rs1800872)					
CC	216	253	1.0		
AC	118	145	1.0	0.7–1.3	
AA	25	24	1.3	0.7–2.4	0.63
IL10-1082 A>G (rs1800896)					
AA	109	141	1.0		
AG	170	191	1.1	0.8–1.6	
GG	80	91	1.1	0.7–1.6	0.59
IL10-3584 T>A (rs1800890)					
TT	116	172	1.0		
AT	150	197	1.1	0.8–1.5	
AA	34	45	1.1	0.7–1.9	0.54
IL13-1069 C>T (rs1800925)					
CC	160	197	1.0		
CT	116	187	0.8	0.5–1.0	
TT	19	28	0.9	0.5–1.6	0.15
IL13 IVS3-24 C>T (rs1295686)					
CC	177	244	1.0		
CT	99	138	1.1	0.8–1.5	
TT	20	30	0.9	0.5–1.7	0.98
IL13 Ex4 + 98 G>A (rs20541)					
GG	174	245	1.0		
AG	100	138	1.1	0.8–1.5	
AA	19	28	1.0	0.5–1.8	0.86
Th1/Th2 genes					
CTLA4 Ex1-61 A>G (T17A) (rs231775)					
AA	89	152	1.0		
AG	153	189	1.4	1.0–1.9	
GG	59	70	1.5	0.9–2.3	0.06
Th3 Genes					
TGF1-509 C>T (rs1800469)					
CC	139	174	1.0		
CT	114	173	0.8	0.6–1.2	
TT	32	45	0.9	0.5–1.4	0.37
TGF1 Ex1-327 T>C (L10P) (rs1982073)					
TT	118	146	1.0		
TC	129	207	0.8	0.6–1.1	
CC	47	70	0.8	0.5–1.3	0.23
TGF1 Ex5-73 C>T (T263I) (rs1800472)					
CC	283	393	1.0		
CT	16	30	0.7	0.4–1.4	
TT	0	1	—	—	0.30

OR, odd ratio; CI, confidence interval; Th1, T helper type 1; Th2, T helper type 2; Th3, T helper type 3.
[1]Numbers do not add up to column totals due to missing genotyping information.
[2]Adjusted for sex, age, education and smoking.

significantly associated with gastric cancer risk (for SNP of rs1389832, OR = 1.6, 95% CI: 1.0–2.6 for A/A carriers relative to G/G carriers, and $P = 0.03$; for rs1494556, OR = 1.7, 95% CI: 1.1–2.7 for C/C carriers relative to A/A carriers, $P = 0.01$; and for rs1494555, OR = 1.5, 95% CI: 1.0–2.4 for G/G carriers relative to A/A carriers, $P = 0.04$). Of the nine Th2 SNPs tested, only *IL5* rs2069812 polymorphism was significantly related to gastric cancer risk. Compared with carriers of the most common C/C genotype, carriers of the T/T genotypes had a reduced risk of borderline significance (OR = 0.5, 95% CI, 0.3–1.0). The *CTLA4* SNP rs231775 was marginally associated with gastric cancer risk. None of the *TGF1* SNPs significantly influenced the risk.

Although none of the tested polymorphisms on *IL13* was significantly associated with gastric cancer risk, haplotype analyses on *IL13* showed that CTG haplotype conferred a decreased risk when compared with the most frequent haplotype (CCG) (Table 2). Haplotype analysis of *IL7R* SNPs showed that the haplotype containing the 'at-risk' alleles at positions rs1389832, rs1494556 and rs1494555, i.e. the ACGC haplotype, resulted in an increased risk when compared with the most frequent haplotype. We did not observe any meaningful haplotype-related results on *IL10*, *LTA* or *IL15* (data not shown). In addition, there was no evidence of

Table 2. Gastric cancer risk by haplotypes in *IL13* and *IL7R*

Haplotype[1]	Case (%)	Control (%)	OR[2]	95% CI
IL13				
CCG	67.4	63.0	1.0	Reference
CTG	9.1	13.0	0.6	0.4–0.9
TCA	6.3	7.1	0.9	0.5–1.3
TTA	17.0	16.5	1.0	0.7–1.3
IL7R				
GAAC	48.1	52.4	1.0	Ref
GAAT	10.3	11.4	1.0	0.7–1.5
ACGC	41.0	35.3	1.3	1.0–1.6

[1]The order of single nucleotide polymorphisms (SNPs) in *IL13* is rs1800925, rs1295686 and rs20541. The order of SNPs in IL7R is rs1389832, rs1494556, rs1494555 and rs7737000.
[2]Adjusted for sex, age, education and smoking.

haplotype effect for SNPs tested in three genes (i.e. *IL4, IL5* and *IL13)* that are located on the same chromosomal region (5q31–33).

Except for *IL7R*, analyses stratified by other risk factors and tumor characteristics, including age, smoking, alcohol consumption, *H. pylori* infection status, Lauren classification, site of tumor origin, tumor grade and tumor stage, produced comparable results (not shown). Carriers of the 'at-risk' *IL7R* alleles at positions rs1389832, rs1494556 and rs1494555 tended to have a more pronounced risk of cancers that originated in the distal subsite and that were poorly differentiated (data not shown).

Gene–gene interaction tests for SNPs from genes in the three pathways, i.e. *IL7R* in the Th1 pathway, *IL5* in the Th2 pathway, *TGF1* in the Th3 pathway, did not reveal any meaningful results, although power was low to detect such effects (data not shown). Nor were significant results observed in interaction tests conducted between the aforementioned SNPs and the Th1 genes *IL1B* and *TNF* previously determined to be associated with gastric cancer risk (data not shown).

DISCUSSION

In this study, we examined SNPs in 10 genes along the Th1, Th2 and Th3 pathways in relation to gastric cancer risk. Of the genes investigated in the Th1 pathway, we found significant associations only with three variants in the *IL7R* gene (rs1389832, rs1494556 and rs1494555). Deficiency in expression of IL7R has been associated with severe combined immunodeficiency (38) and several polymorphisms have been linked with multiple sclerosis (39). However, polymorphisms in *IL7R* have seldom been studied with respect to cancer risk. To our knowledge, though a non-Hodgkin lymphoma (NHL) case–control study found no association with rs1494555 polymorphism (40), this is the first study to investigate whether *IL7R* genetic variants affect the risk of a solid cancer. Replication of our findings in larger studies with more complete coverage of this gene is needed before we can draw further inference about the role of this gene in gastric carcinogenesis.

LTA rs909253, which affects expression of LTA (41), has been linked with immune and inflammatory-related diseases such as atopic asthma (42) and celiac disease (43). However, null associations with rs909253 have been reported for NHL (40,44), gastric cancer (45) and colorectal cancer (46). Increased levels of IL15 have been implicated in rheumatoid arthritis (47), but *IL15* rs10833 was not associated with NHL (40). Consistent with previous studies of cancer, we did not observe an effect of either *LTA* or *IL15* SNPs or haplotypes on gastric cancer risk.

Of the polymorphisms in Th2 genes examined, only one *IL5* polymorphism (rs2069812) was significantly associated with gastric cancer risk. Although the biological function of rs2069812 is unclear and, to our knowledge, no data on its association to cancer risk are available, it has been associated with immune response-related diseases, such as atopic bronchial asthma and blood eosinophilia (48,49). These observations raise the possibility that this polymorphism plays a role in the etiology of gastric cancer.

Although *IL13* SNPs were not associated with gastric cancer risk individually, a significant reduction in risk was observed for the CTG haplotype when compared with the most common haplotype (CCG) in our study. However, as the T allele at rs1295686 was not associated with gastric cancer risk in the TTA haplotype, the observed result with the CTG haplotype may be due to linkage disequilibrium with an unknown variant. Among the three SNPs studied, rs20541 has been associated with asthma and type 1 diabetes in several populations (50,51), but no data on cancer risk are available.

The *IL4* rs2243250 promoter polymorphism is known to increase transcriptional activity of *IL4* (52) and has been associated with asthma (52) and a number of inflammation-related diseases (53,54). However, two gastric cancer studies that examined this promoter polymorphism have reported null associations (35,55), which is in agreement with our findings.

The *IL6* promoter SNP (rs1800795) was associated with increased IL6 production (26). To date, this polymorphism has not been associated with chronic gastritis, inflammatory process in gastric mucosa or gastric cancer (35,56,57). This study did not observe an association with gastric cancer risk.

Three *IL10* promoter SNPs (rs1800896, rs1800871 and rs1800872) have been linked with differences in mucosal IL10 expression in the course of chronic *H. pylori* infection (58). However, the associations between these polymorphisms and gastric cancer risk have been inconsistent (35,59). In this study, we did not observe an association with gastric cancer risk.

CTLA4 rs231775 polymorphism has been associated with Type 1 diabetes (60), Crohn's disease (61) and Graves'

disease (62). It has also been implicated in the etiology of gastric mucosa-associated lymphoid tissue lymphoma (63), colorectal cancer (64) and NHL (65), although another study has reported a null finding with NHL (40). In this study, we found an association of borderline significance of this gene with gastric cancer risk.

TGF1 is the only Th3 gene we examined in this study. There has been much focus on the role of this gene in carcinogenesis. A number of cancers are characterized by elevated TGF1 production, including prostate, gastric, colorectal, breast (66). Several polymorphisms are known to alter the expression or function of TGF1, including rs1800469 and rs1982073 (67). Despite the strong biologic rationale, the results of association studies between *TGF1* SNPs and cancer outcomes are inconsistent (68,69). We found no significant associations between *TGF1* polymorphisms and gastric cancer risk in this study.

The present study is based on a relatively large study of gastric cancer conducted in a high risk population. The fact that this study was population-based and had high-participation rates strengthens its findings. Misclassification was minimal due to the high reproducibility and accuracy of genotyping. However, the gene coverage of our SNP selection was limited. The observed associations between *IL5* rs2069812 and *IL7R* rs1389832, rs1494556 and rs1494555 and gastric cancer risk could be due to the influence of other alleles, which are in linkage disequilibrium with these polymorphisms. These observations could also be due to chance as a result of multiple comparisons. Our finding by *H. pylori* infection status could be false because of (i) the small number of *H. pyori*-positive controls (79 positive and 336 controls) and *H. pylori*-negative cases (227 positive and 73 controls) and minor allele carries, our stratified analysis by *H. pylori* status had very limited power and (ii) being case–control study, our information on the prevalence of *H. pylori* infection among cases may not be accurate owing to clearance of *H. pylori* colonization with tumor progression. Still, our findings in these key immune function genes in relation to gastric cancer risk provide intriguing leads that can be further investigated in larger studies. In conclusion, the present study suggests that the *IL5* rs2069812 and *IL7R* rs1389832, rs1494556 and rs1494555 polymorphisms may contribute to the etiology of gastric cancer. Further studies will be needed to analyze additional SNPs for the full coverage of the genes across different populations.

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Conflict of interest statement

None declared.

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