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CD14-159C/T and *TLR9*-1237T/C polymorphisms are not associated with gastric cancer risk in Caucasian populations

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Abstract

Host genetic factors play an important role in modifying the risk of human disease, including cancers of the upper gastrointestinal tract, with increasing interest in Toll-like receptor (TLR) signaling and the impact of genetic polymorphisms in these systems. The *CD14*-159C/T and the *TLR9*-1237T/C promoter polymorphisms have previously been shown to be associated with various inflammatory conditions including *Helicobacter pylori*-induced gastritis in Caucasian populations. In this study, we assessed the association of these two functional single nucleotide polymorphisms with gastric cancer in two independent Caucasian population-based case–control studies of upper gastrointestinal tract cancer, initially in 312 noncardia gastric carcinoma cases and 419 controls and then in 184 noncardia gastric carcinomas, 123 cardia carcinomas, 159 esophageal cancers, and 211 frequency-matched controls. Odds ratios were computed from logistic models and adjusted for potential confounding factors. No significant association was found between the *CD14*-159C/T and the *TLR9*-1237T/C promoter polymorphisms and increased risk of gastric cancer. Neither single nucleotide polymorphism has been assessed in a Caucasian gastric cancer case–control study before; although the *CD14*-159C/T polymorphism has been reported to show no apparent association with *H. pylori*-related gastric malignancy in a Taiwanese Chinese population. In conclusion, although our earlier preliminary studies suggested that the *CD14*-159C/T and the *TLR9*-1237T/C promoter polymorphisms increase the risk of precancerous outcomes, they do not seem to increase the risk of gastric cancer itself. This discrepancy merits further examination.

Keywords

gastric cancer; *Helicobacter pylori*-induced disease; innate immunity; polymorphisms; Toll-like receptor signaling pathways

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Introduction

Helicobacter pylori is the most important acquired risk factor for gastric cancer and its interaction with the innate immune response, especially Toll-like receptors (TLRs) is key to clinical outcome (Hold *et al.*, 2007). A single nucleotide polymorphism in the lipopolysaccharide (LPS) recognition receptor complex –such as TLR4 (*TLR4*+896A/G, rs4986790) has been shown to be a risk factor at various stages of *H. pylori*-induced gastric carcinogenesis (Hold *et al.*, 2003, 2006, 2007; Kato *et al.*, 2007). Another variant within the LPS recognition receptor complex, the *CD14*-159C/T (rs2569190), is situated close to a binding site for the Sp1 transcription factor, which is critical for CD14 expression. Carriage of the variant *CD14*-159 T allele is associated with increased soluble CD14 expression (Baldini *et al.*, 1999) and inversely associated with levels of total serum IgE (Baldini *et al.*, 1999; Koppelman *et al.*, 2001) and has been studied in various inflammatory conditions (Hubacek *et al.*, 1999; Arnott *et al.*, 2004; Torok *et al.*, 2004).

We have also studied a polymorphism within another TLR involved in bacterial recognition, such as TLR9 (*TLR9*-1237T/C, rs574383). In a work published in abstract form, we found that the polymorphism was associated with the development of premalignant gastric changes (Hold *et al.*, 2006). TLR9 is responsible for initiating responses to bacterial CpG DNA in human inflammatory cells (Hemmi *et al.*, 2000; Bauer *et al.*, 2001), with upregulation of *TLR9* mRNA expression noted in murine macrophages in response to bacterial LPS (An *et al.*, 2002). Within the gastric environment, the presence of *H. pylori* has been shown to induce expression of TLR9 on gastric epithelial cells, and in-silico analysis of the *TLR9*-1237T/C promoter polymorphism indicates that the single nucleotide polymorphism (SNP) lies within a putative nuclear factor κB-binding site and is therefore a biologically plausible candidate SNP (Schmausser *et al.*, 2004). The aim of our study was to assess the effect of the *TLR9*-1237 T/C and *CD14*-159C/T polymorphisms on the risk of upper gastrointestinal cancer in Caucasians.

Materials and methods

Study populations

The *CD14*-159C/T and *TLR9*-1237T/C SNPs were genotyped in two previously described independent Caucasian population-based case–control studies of upper gastrointestinal tract cancer (El Omar *et al.*, 2000). The first was a gastric cancer case–control study derived from a Caucasian population in Warsaw, Poland in which there were DNA samples available from 327 noncardia gastric adenocarcinoma patients and 406 controls (Chow *et al.*, 1999). The second was a multicenter esophageal and gastric cancer study conducted in three geographic areas of the United States with population-based tumor registries (Gammon *et al.*, 1997); DNA samples were available from 306 patients with gastric adenocarcinoma (122 cardia and 184 noncardia, 90% Caucasian), 159 patients with esophageal cancer (52 with squamous cell carcinoma and 107 with adenocarcinoma, 90% Caucasian), and 211 population controls (94% Caucasian). The institutional review boards of the participating centers approved the study, and written informed consent was obtained from all participants.

Genotype assays

CD14-159C/T and *TLR9*-1237T/C SNPs were genotyped with Taqman assays using minor groove binder AQ2probes. For *CD14*-159C/T, forward primer 5′ CTAGATGCCCTGCAGAATCCTT-3′ and reverse primer 5′ CCCTTCCTTTCCTGGAAATATTGCA-3′ were used along with wild-type probe VIC: CTGTTACGGCCCCCCT and variant allele probe FAM: CTGTTACGGTCCCCCT. For *TLR9*-1237T/C, forward primer 5′-CAGAGACATAATGGAGGCAAAGGA-3′ and reverse primer 5′-GCCTTGGGATGTGCTGTTC-3′ were used along with wild-type probe VIC:

CTGCCTGAAAACT and variant allele probe FAM: TCTGCCTGGAAACT. Sequencing of selected genotypes was carried out to validate Taqman results.

Statistical analyses

Hardy–Weinberg equilibrium of alleles at individual loci was assessed by χ^2 statistics. Odds ratios (ORs) with Cornfield 95% confidence intervals were computed by logistic regression using STATA version 7.0 software (STATA Press, College Station, Texas, USA). ORs for each cancer were adjusted for age (categorized as younger than 50, 50–59, 60–69, and 70 years or older), sex, and race (categorized as white and all other). Estimates of study power were assessed using Quanto (http://hydra.usc.edu/gxe/). The Polish gastric cancer study had 80–90% power to detect ORs of 1.6 or greater at a 5% significance level, and 50–60% power to detect ORs as low as 1.4.

Results

In both control populations, the alleles of *CD14*-159C/T and *TLR9*-1237T/C were in Hardy–Weinberg equilibrium, with nonsignificant χ^2 values. The allele frequencies in the control populations were similar to other documented Caucasian studies, *CD14* 159C/T variant allele (43–50%), *TLR9*-1237T/C variant allele (11–16%) (Tables 1 and 2). Neither SNP was associated with risk for gastric cancer in the Polish gastric cancer study (Table 1) nor with the various types of upper gastrointestinal cancer in the US-based case–control study (Table 2) in analyses adjusted for age, sex, and race (only Caucasians are reported as other ethnic groups were very small). The additional models, adjusted for other factors, gave qualitatively similar results (data not shown).

Discussion

As a result of the known and putative functional effects of the *CD14*-159C/T and *TLR9*-1237T/C promoter polymorphisms, respectively, and our preliminary findings relating to their association with the development of *H. pylori*-induced premalignant gastric changes (Hold *et al.*, 2006), it was important to assess their relevance to gastric cancer. Neither SNP has been assessed in a Caucasian gastric cancer case–control study before; although the *CD14*-159C/T polymorphism has been reported to show no apparent association with *H. pylori*-related gastric malignancy in a Taiwanese Chinese population (Wu *et al.*, 2006). The *CD14*-159T allele has, however, been associated with increased risk of intestinal metaplasia in a Venezuelan population (Kato *et al.*, 2007). Intestinal metaplasia, however, may not be the most appropriate surrogate marker of gastric cancer risk, as malignant potential depends on the subtype and this is often not defined in genetic association studies. Yet, in our study, neither SNP seemed to be risk factors for gastric cancer in either of the large Caucasian study populations tested. Ethnic-specific host susceptibility in gastric cancer development has been reported previously (Canedo *et al.*, 2008).

On the basis of these findings, it is possible that the *CD14*-159C/T and *TLR9* 1237T/C polymorphisms are only risk factors at the early stages of the disease process. They may be relevant in defining the host immune response to *H. pylori* infection, but it would appear that they do not determine subsequent events in carcinogenic progression. Interestingly, variants in other genes that play a critical role in *H. pylori*-induced gastric cancer have also been identified as risk factors in the precursor stages of the disease process but not at the cancer stage (Savage *et al.*, 2006).

It must also be considered that the *CD14*-159C/T and *TLR9*-1237T/C polymorphisms are not relevant markers in the study populations tested. Alternatively, it is possible that a more detailed assessment of the genes with more markers may show an association. The fact that two

reasonably sized independent gastric cancer case–control studies have, however, failed to show a positive finding with these markers suggests that the results are genuine and that an association was not missed owing to low study power.

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Table 1

Genotype frequencies and adjusted odds ratios (and Cornfield 95% confidence intervals) for the *CD14*-159C/T and *TLR9*-1237T/C polymorphisms in Polish gastric cancer cases and controls

Locus	Genotype	Controls, *n* (%)	Cases, *n* (%)
CD14-159C/T	C/C	131 (34)	110 (34)
	C/T	176 (45)	134 (41)
	T/T	82 (21)	83 (25)
Adjusted OR (95% CI)[a]	C/C vs. C/T+T/T		1.0 (0.7–1.4)
TLR9-1237T/C	T/T	316 (78)	261 (80)
	T/C	85 (21)	58 (18)
	C/C	5 (1)	7 (2)
Adjusted OR (95% CI)[a]	T/T vs. T/C+C/C		0.9 (0.6–1.3)

CI, confidence interval; OR, odds ratio

[a]Odds ratios adjusted for age and sex.

Table 2

Genotype frequencies and adjusted odds ratios (and Cornfield 95% confidence intervals) for the *CD14*-159C/T and *TLR9*-1237T/C polymorphisms in US patients with different types of upper gastrointestinal cancer and controls

			Esophageal cancer		Gastric cancer	
Locus	**Genotype**	**Controls, *n***	**Squamous, *n* (%)**	**Adeno, *n* (%)**	**Cardia, *n* (%)**	**Noncardia, *n* (%)**
CD14-159C/T	C/C	52 (25)	14 (27)	36 (34)	38 (31)	53 (29)
	C/T	108 (51)	27 (53)	47 (44)	53 (44)	94 (51)
	T/T	51 (24)	10 (20)	24 (22)	31 (25)	37 (20)
Adjusted OR (95% CI)[a]	C/C vs. C/T+T/T		1.2 (0.6–2.5)	0.6 (0.4–1.0)	0.7 (0.4–1.2)	0.8 (0.5–1.3)
TLR9-1237T/C	T/T	149 (71)	40 (77)	77 (76)	85 (71)	139 (78)
	T/C	57(27)	7 (13)	22 (22)	31 (26)	38 (21)
	C/C	4 (2)	5 (10)	2 (2)	4 (3)	1 (1)
Adjusted OR (95% CI)[a]	T/T vs. T/C+C/C		0.7 (0.4–1.5)	0.8 (0.4–1.31)	1.1 (0.7–1.8)	0.6 (0.4–1.0)

CI, confidence interval; OR, odds ratio

[a]Odds ratios adjusted for age, sex, and race.