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Genetic variation in *C20orf54, PLCE1* and *MUC1* and risk of upper gastrointestinal cancers in Caucasian populations

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Abstract

Recently, two large genome wide association studies, conducted in Chinese populations, reported associations between upper gastrointestinal (GI) cancer and the rs2274223, rs13042395 and rs4072037 polymorphisms in *PLCE1, C20orf54* and *MUC1* respectively. We set out to determine whether similar associations existed for Caucasian populations.

We genotyped two population-based, case-control studies of upper GI cancer; the first included 290 gastric cancer cases and 376 controls; the second study included 306 gastric cancer cases, 107 oesophageal adenocarcinoma cancer cases, 52 oesphageal squamous cell cancer cases, and 211 controls. Odds ratios (OR) and 95% confidence intervals (CI) were computed from logistic models and adjusted for confounding variables.

The rs4072037 polymorphism in *MUC1* was associated with a reduced risk of gastric cancer of intestinal histological type (OR 0.4; 95% CI 0.2–0.9), and a reduced risk of oesophageal squamous cell cancer (OR 0.5; 95% CI 0.2–1.0), but not oesphageal adenocarcinoma. Likewise, rs2274223 in *PLCE1* was associated with a reduced risk of oesophageal squamous cell cancer (OR 0.5; 95% CI 0.3–1.0) but not oesphageal adenocarcinoma. We observed no association between rs13042395 in *Corf54* and risk of gastric or oesphageal cancer in either of the two studies.

Our findings for rs4072037 and gastric cancer risk are in keeping with one previous report for a Caucasian population. To the best of our knowledge this is the first study to report an association between rs2274223 and rs4072037 and risk of oesophageal squamous cell carcinoma in a Caucasian population.

Introduction

We have witnessed great advances in our understanding of the genetic basis of gastric and oesphageal cancers (upper gastrointesinal (GI) cancers). This has been the result of well-powered, genome wide association studies (GWAS) exploring the relationship between a large number of single nucleotide polymorphisms (SNP) and disease predisposition.

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Recently, two large GWAS, conducted in Chinese populations, reported associations between gastric cancer (GC) and oesphageal squamous cell carcinoma (OSCC), and a number of previously unknown disease-susceptibility loci at 1q22, 10q23 and 20p13 (Abnet, Freedman et al. 2010, Wang, Zhou et al. 2010). Of particular interest were three SNP: rs2274223 (G>A) in *PLCE1* at 10q23 and rs13042395 (T>C) in *C20orf54* at *20p13*, which were shown to increase risk for GC and OSCC, and rs4072037 (G>A) in *MUC1* at 1q22, which was shown to confer protection from GC. We set out to determine whether similar associations existed for Caucasian populations.

Materials and methods

Study groups

We genotyped two, Caucasian, population-based, case-control studies of upper GI cancer: the first included 290 GC cases (predominantly non-cardia GC) and 376 controls derived from a population in Warsaw, Poland (Chow, Swanson et al. 1999); the second study, derived from three distinct geographical areas of the United states holding population based cancer registries, included 122 cardia GC cases, 184 non-cardia GC cases, 107 oesophageal adenocarcinoma cancer cases (OACC), 52 OSCC), and 211 controls (Gammon, Ahsan et al. 1997).

Genotyping

DNA samples were genotyped by real-time PCR allelic discrimination using the ABI 7900HT Fast Sequence Detection System (Applied Biosystems, Foster City, CA). Predesigned TaqMan SNP genotyping assays were employed, utilizing minor groove binding probes 5′-labeled with VIC or FAM fluorophores to detect the allelic variants in rs2274223, rs13042395, rs4072037 (Applied Biosystems). Following analysis of real time data, genotyping calls were possible in 98% of samples for the rs13042395 and rs4072037 SNPs and >96% for rs2274223SNP.

Statistics

Hardy–Weinberg equilibrium (HWE) of alleles was assessed at the polymorphic loci by χ^2 statistics. Odds ratios (OR) with Cornfield 95% confidence intervals (CIs) were computed by logistic regression, and adjusted for age and sex using STATA software (version 7.0; STATA Press, College Station, TX).

Results

For all three SNP, the distribution of alleles was in HWE, with non-significant χ^2 values in both control groups.

The rs4072037 polymorphism (G>A) in *MUC1* was inversely associated with risk of intestinal GC, and OSCC, in the US study group. For intestinal type GC, the association was observed for heterozygotes, with an OR of 0.4 (95% CI 0.2–0.9), and in a dominant model (OR 0.4 (95% CI 0.2–0.9)). Similarly for OSCC, the association was observed for heterozygotes (OR 0.4 (95% CI 0.2–0.9)) and in dominant model (OR 0.5 (95% CI 0.2–1.0)).

The rs2274223 polymorphism (G>A) in *PLCE1* was inversely associated with risk of OSCC in the US study group. The association was significant only in heterozygotes (OR 0.5; 95% CI 0.2–1.0) (see table 2). No associations were observed between the rs2274223 polymorphism and GC in either the Polish or US study groups.

No associations were observed between the rs13042395 polymorphism in *C20orf54* and risk upper GI cancer in either study population (results not shown).

Discussion

The rs2274223 polymorphism in *PLCE1* was identified as a risk factor for GC and OSCC in Han Chinese populations by two contemporaneous independent GWAS (Abnet, Freedman et al. 2010, Wang, Zhou et al. 2010).

The association with GC was subsequently reproduced in another study in Han Chinese (Zhang, Jin et al. 2011). In Caucasian populations, associations have been reported between rs2274223 and risk for non-oropharyngeal squamous cell carcinoma of the head and neck (Ma, Wang et al. 2011). Our finding of an association with OSCC is in the opposite direction to those reported in the literature; such affects themselves are not, however, novel, with opposing effects observed for other SNPs reported for different tumour sites (Lochhead, Frank et al.). Interestingly, the results of a study in a Chinese population suggest that rs2274223 confers a survival advantage in GC (Luo, Gao et al. 2011).

PLCE1 encodes an enzyme that catalyses the hydrolysis of phosphatidylinositol-4,5-bisphosphate, generating the secondary messengers inositol 1,4,5-triphosphate and diacylglycerol, which participate in cell growth, differentiation and gene expression (Bunney, Baxenale et al. 2009). PLCE1 is regulated by small GTPases of the Ras, Rap and Rho families, and contains a guanine nucleotide exchange factor domain for Ras-like small GTPases at its N-terminus, and two Ras-binding domains at its C-terminus (Bunney, Baxenale et al. 2009, Kelley, Reks et al. 2001, Wing, Bourdon et al. 2003). The latter two domains bind directly to H-ras (Kelley, Recks et al. 2001). PLCE1 also interacts with IQ-domain GTPase-activating protein 1 (IQGAP1) (Hinkes, Wiggins et al. 2006). IQGAP1 has important roles in angiogenesis, and is expressed in endothelial cells where it binds VEGFR2, an important factor for endothelial cell rearrangement and migration (Johnson, Sharma et al. 2009).

With little known about rs2274223 prior to the aforementioned GWAS, no functional data exist. In colorectal cancer (CRC), *PLCE1* expression is significantly down-regulated compared to the normal colonic mucosa, with increasing suppression of *PLCE1* correlated with advancing tumour stage (Danielsen, Cekaite et al. 2011). In a CRC cell line, overexpression of *PLCE1* was found to inhibit cell proliferation and promote apoptosis (Wang, Zhou et al. 2011). These findings suggest that *PLCE1* functions as a tumour suppressor gene; however, tumour promoting effects for PLCE1 have also been reported. In the APC min/+ mouse, knockout of *PLCE1* was associated with resistance to tumour development through attenuation of angiogenesis and tumour associated inflammation (Li, Edamatsu et al. 2009). Similar pro-inflammatory actions have been observed in skin carcinogenesis (Bai, Edamatsu et al. 2004).

The finding that rs4072037 in *MUC1* protects against GC has been validated in Han Chinese (Zhang, Jin et al. 2011, Shi, Hu et al. 2011), Japanese, South Korean (Saeki, Saito et al. 2011), and Caucasian populations (Jia, Persson et al. 2010).

The mucin, MUC1, is a highly glycosylated trans-membrane glycoprotein that is a major component of the viscous mucous layer protecting epithelial surfaces. Functional studies have shown that rs4072037, in the *MUC1* gene, regulates alternative splicing of the second exon, and modifies the transcriptional activity of the promoter (Saeki, Saito et al. 2011). However, the mechanism by which rs4072037 contributes to GC development has yet to be elucidated.

More recently, it has been shown that MUC1 participates intracellularly in the activation of the NF-κB, leading to up-regulation of cytokines IL-6 and TNF-α (Cascio, Zhang et al. 2011). This is an interesting and novel observation, given that prognosis in both GC and OSCC is inversely associated with *MUC1* expression (Utsunomiya, Yonezawa et al. 1998, Ye, Yan et al. 2011). Moreover, *MUC1* expression in OSCC upregulates the expression of matrix metalloproteinase 13, a protein which is highly expressed in tumours of patients with metastatic OSCC, and which is indirectly regulated by NF-κB through the production of pro-inflammatory cytokines (Ye, Yan et al. 2011). As such, this provides a potential mechanism by which tumours can promote inflammation and tumour development through *MUC1* expression.

Despite being a risk factor for upper GI cancer in Asians, our data do not support a role for rs13042395 and upper GI cancer risk in Caucasians. However, as the minor allele is at such a low frequency in our study populations, we cannot exclude the possibility that we were unable to detect an association due to lack of power.

rs2274223 has been reported by others to be in linkage disequilibrium (LD) with *NOC3L* and another SNP in *PLCE1*, rs3203713, (Zhang, Jin et al. 2011). In addition, rs4072037 has been reported to be in LD with the small allele of the variable number of tandem repeats in the second intron of *MUC1* (Jia, Persson et al. 2010). With no functional data, it is possible that the associations we observed are due to linkage with these or another functional polymorphism in the same region.

The fact that our observed associations were most apparent in heterozygotes is worth some consideration. While this could be due to a heterozygote advantage, it is likely that these findings reflect power limitations by small number of homozygote minor allele cases in the study groups. Indeed, in support of this explanation, our dominant models are in accordance with the observed associations for heterozygotes.

Given that we considered this an exploratory study, we have not corrected for multiple comparisons, and cannot exclude the possibility that the significant findings have arisen by chance. Furthermore, the small number of OSCC cases in the US study results in large confidence intervals for these ORs. However, we believe that the novelty and potential importance of our findings merits their validation in additional Caucasian studies of upper GI malignancy.

In conclusion, this study has added to our knowledge of the complex genetic interactions that define upper GI cancer risk in Caucasians. These findings may give insight into the molecular pathogenesis of upper GI malignancy and, in the future, may contribute to individual risk stratification for prevention and screening.

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Table 1

Association between the rs4072037 polymorphism in *MUC 1* and risk of gastric and oesophageal cancer

US STUDY Gastric Cancer	**AA**	**AG**	**GG**	**Dominant**	**Recessive**
Non-Cardia	44/32 1.0 (Ref)	91/118 0.6 (0.3–1.1)	54/57 0.8 (0.4–1.4)	0.7 (0.4–1.1)	1.1 (0.7–1.7)
Cardia	29/32 1.0 (Ref)	**53/118** **0.5 (0.3–0.9)**	40/57 0.8 (0.4–1.6)	0.6 (0.3–1.1)	1.3 (0.8–2.1)
Non-Cardia Diffuse Type	15/32 1.0 (Ref)	39/118 0.8(0.4–1.4)	28/57 1.0 (0.5–2.4)	0.8 (0.4–1.7)	1.4 (0.8–2.4)
Non-Cardia Intestinal Type	17/32 1.0 (Ref)	**25/118** **0.4 (0.2–0.9)**	16/57 0.5 (0.2–1.3)	**0.4 (0.2–0.9)**	1.0 (0.5–2.0)
Oesphageal cancer					
Adenocarcinoma	22/32 1.0 (Ref)	44/118 0.5 (0.3–1.1)	41/57 1.0 (0.5–2.2)	1.0 (0.6–1.6)	1.6 (1.0–2.8)
Squamous Cell	15/32 1.0 (Ref)	**21/118** **0.4 (0.2–0.9)**	16/57 0.6 (0.2–1.5)	**0.5 (0.2–1.0)**	1.2 (0.6–2.4)

Key: Per genotype, dominant and recessive models are shown with OR and 95% CI; x/y=cases/ controls; results in bold denote P<0.05.

Table 2

distribution of rs2274223 and risk of gastric and oesophageal cancer

POLISH STUDY Gastric Cancer	AA	AG	GG	Dominant	Recessive
All sites	107/154 1.0 (ref)	138/166 1.2 (0.8–1.7)	44/56 1.1 (0.7–1.8)	1.2 (0.9–1.6)	1.0 (0.7–1.6)
Intestinal type	49/154 1.0 (ref)	1.1 (0.8–1.7) 87/166	31/56 1.2 (0.7–2.1)	1.0 (0.5–1.9)	1.1 (0.7–1.8)
Diffuse type	190/154 1.0 (ref)	21/166 1.0 (0.5–2.0)	8/56 1.1 (0.4–2.8)	1.1 (0.8–1.7)	1.1 (0.4–2.6)
Antrum	63/154 1.0 (ref)	57/166 1.5 (0.9–2.5)	16/56 1.3 (0.6–2.6)	1.3 (0.7–2.4)	1.0 (0.5–1.9)
US STUDY Gastric Cancer					
Non-Cardia	80/86 1.0 (ref)	89/107 0.9 (0.6–1.4)	15/17 0.9 (0.4–2.2)	0.9 (0.6–1.4)	1.0 (0.5–2.2)
Cardia	52/86 1.0 (ref)	61/107 0.9 (0.6–1.5)	9/17 0.9 (0.3–2.3)	0.9 (0.6–1.5)	0.9 (0.3–2.2)
Non-Cardia Diffuse Type	34/86 1.0 (ref)	38/107 0.9 (0.5–1.6)	9/17 1.3 (0.5–3.5)	1.0 (0.6–1.7)	1.4 (0.5–3.5)
Non-Cardia Intestinal Type	26/86 1.0 (ref)	26/107 0.8 (0.4–1.6)	6/17 1.2 (0.3–3.5)	0.9 (0.5–1.6)	1.3 (0.4–307)
Oesphageal Cancer					
Adenocarcinoma	44/86 1.0 (ref)	50/107 0.9 (0.5–1.5); 0.719	13/17 1.5 (0.6–3.6); 0.328	1.0 (0.6–1.6); 0.977	1.6 (0.7–3.6); 0.244
Squamous Cell	30/86 1.0 (ref)	**18/107** **0.5 (0.2–1.0)**	4/17 0.7 (0.2–2.3)	**0.5 (0.3–1.0)**	0.9 (0.2–3.1)

Key: Per genotype, dominant and recessive models are shown with OR and 95% CI; x/y=cases/ controls; results in bold denote P<0.05.